



QT 25 C325L 1916

06920380R



NLM 05047656 3

NATIONAL LIBRARY OF MEDICINE

SURGEON GENERAL'S OFFICE  
LIBRARY.

ANNEX

ANNEX

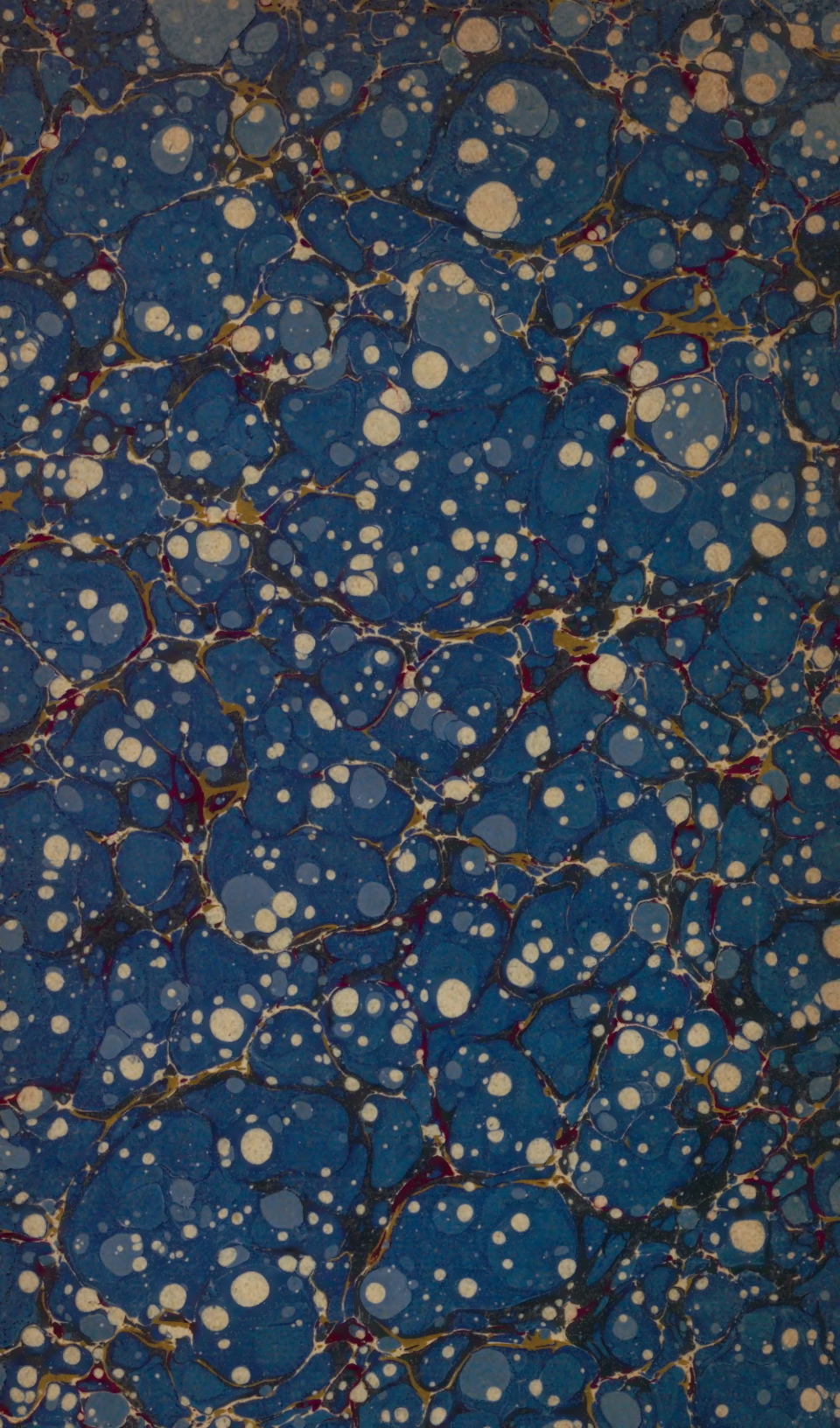
Section

No. 113,  
W. D. S. G. O.

No. 224995

3-513



















103676  
Wang  
205

B313-716-500

LABORATORY EXERCISES

IN

PHYSIOLOGY

---

ARRANGED FOR THE USE OF MEDICAL STUDENTS

BY

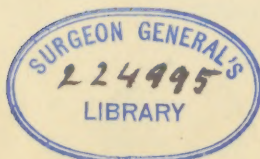
✓  
WILLIAM S. CARTER, M. D.,

PROFESSOR OF PHYSIOLOGY IN THE UNIVERSITY OF TEXAS,  
GALVESTON, TEXAS.

---



AUSTIN, TEXAS  
A. C. Baldwin & Sons, Printers  
1916





QT

25

C325L

1916

Copyright 1916

by

W. S. Carter ✓

Transferred from the Library  
of Congress under Sec. 59,  
Copyright Act of Mar. 4, 1909

OCT 12 1916 ✓

\$1.00 ✓

©CLA445070 ✓

no 2



# Laboratory Exercises in Physiology

---

Arranged for the Use of Students in the School of Medicine,  
University of Texas.

---

By

**WILLIAM S. CARTER, M. D.,**  
Professor of Physiology.

---

## THE BLOOD.

**1. The Reaction of Blood:** Put a drop of fresh defibrinated dog's blood on neutral glazed litmus paper and after 20 or 30 seconds wash the blood off with distilled water. The litmus paper can be glazed by dipping it into a solution of neutral gelatin and allowing it to dry.

A faint blue color shows that fresh blood is slightly alkaline. The same reaction is obtained with blood serum. If the serum is clear and free from hemoglobin it is not necessary to wash it off with water as the serum does not obscure the change of color with litmus.

In the same way take the reaction of fresh human blood obtained from the finger by a sterile needle after wiping the skin with cotton wet with alcohol. Record the result of each observation.

**2. The Potential Alkalinity of Blood Serum:** Take 10 c. c. of clear serum free from hemoglobin in each of two small beakers or in test-tubes. Add a few drops of phenolphthalein to one and a few drops of methyl orange to the other. Observe that neither changes color. From the point of view of physical chemistry





the blood may be regarded as practically a neutral fluid, since the faint alkalinity shown with litmus is not enough to change the phenolphthalein.

Now cautiously add from a graduated burette or pipette a  $\frac{N}{10}$  NaOH sol. to the serum containing phenolphthalein, and in the same way, titrate  $\frac{N}{10}$  HCl sol. into the serum containing the methyl orange. Observe and record in each case the exact amount used until a permanent red color is produced.

Now take two portions of 10 c. c each of distilled water and add one of the indicators used above to each. Titrate in the same way and record the result. Contrast this with the amount used with blood serum. Explain the difference.

Can the power of the blood to neutralize acids be determined by the ordinary methods of titration? How does the alkali exist in the blood? Does it give to the blood a distinct absolute alkalinity physically or a potential alkalinity physiologically?

**3. The Specific Gravity of Blood:** Mix one part of chloroform with three parts of benzol in a hydrometer cylinder and add a little of one or the other fluid until a mixture is obtained which after thorough mixing has a specific gravity of 1.050 or 1.055 as determined by a hydrometer.

After stirring some defibrinated blood in a beaker, draw a little up in a pipette with a narrow point. Allow a drop of the blood to escape into the fluid and observe whether it sinks to the bottom or rises to the top. If the blood is heavier, add chloroform and mix thoroughly until the blood remains suspended in the fluid; if the blood is lighter, add benzol until the specific gravities are equalized and the drop of blood remains suspended in the mixture after it has been stirred up. Now take the specific gravity of the mixture with the hydrometer. This corresponds to the specific gravity of the blood.

Repeat the observation, using a drop of fresh human blood, and record the specific gravity.

How would the condition of hydremia, or an excess of water in the blood, influence the specific gravity?

**4. The Coagulation Time:** The blood usually clots in 3 to 5 minutes after it is shed, and the time required for clotting is taken as an index of the coagulability of the blood. Vari-



ous coagulometers have been devised, but the results vary considerably with the method used. The most important factor is the temperature at which the blood is kept after it is removed from the body.

The most convenient coagulometer consists of a series of small loops of uniform size in a platinum wire (Biffi-Brooks' method). These loops are filled with drops of blood and the glass rod holding the wire is gradually pushed through a perforated stopper, so that one loop is submerged in physiological salt solution every half minute until the blood remains clotted in the loop and does not mix with the solution. The fluid is kept at the bodily temperature, but there is no uniformity in the temperature of the air in the flask or bottle over the physiological salt solution. This constitutes the greatest defect in this method and explains why considerable variations occur in repeated observations on the same blood.

Perhaps the most satisfactory coagulometer consists of a glass pipette made into a small piston syringe to hold a drop of blood submerged in water until it clots.

The outer glass tube is drawn out to a pipette point about three inches long and having a lumen of less than 1 mm. at the end. Another tube of suitable size is drawn out with a bulbous end so that a piece of rubber tubing tied over it serves as a piston when it is moistened.

A flask of water at 38°C is prepared with a stopper having two perforations and a thermometer in one of them.

The skin of the finger is cleansed thoroughly and a large drop of blood obtained with a sterile needle, noting the exact time when the drop begins to form.

As quickly as possible the blood is drawn up into the clean, dry pipette by cautiously withdrawing the piston so that all the blood is taken up avoiding any mixture with air bubbles.

The pipette is then immersed in the water up to the level of the top of the blood and every half minute a very small drop of blood is expelled into the water by gently pushing the piston down. This is continued until the blood can not be expelled, or until a clot forms and can be seen floating in the water after some of the blood has been forced out of the pipette. Either may be taken as the end reaction when the fluidity of the blood





ceases and the time that elapsed from the drawing of the blood is the coagulation time.

**5. The Amount of Sodium Chloride in Solution Which Is Isotonic with the Erythrocytes:** Two graduated centrifuge tubes of the same capacity are filled with exactly the same amount of defibrinated blood and centrifugalized until the corpuscles sink to the lowest level. The exact volume of sedimented corpuscles is noted in each tube.

Then the serum is pipetted off of one tube and replaced with 0.9 per cent NaCl sol. The corpuscles are stirred up in both tubes and centrifugalized again until no further sedimentation occurs. The volume of corpuscles in each tube is again observed and recorded.

The 0.9 per cent NaCl sol. is pipetted off and replaced with 1.0 per cent NaCl. The corpuscles are stirred up in both tubes and centrifugalized again. The volume of corpuscles in each tube is again noted and compared with the previous readings.

The 1.0 per cent NaCl is pipetted off and replaced with 0.8 per cent NaCl sol. The corpuscles are stirred up in both tubes and centrifugalized as before. Observe and record the result. The observation may be repeated with 0.7 per cent NaCl sol.

Compare the results with the different solutions of sodium chloride and state which ones are hypertonic, isotonic and hypotonic, respectively.

Explain why the volume of the corpuscles is changed by hypertonic and hypotonic solutions, respectively.

**6. The Osmotic Resistance of Erythrocytes:** Take a series of ten test-tubes and from a graduated burette or pipette run into the first 6 c. c. of 1 per cent NaCl sol.: into the second 5.8 c. c.; into the third 5.6 c. c., and so on to the end of the series, always making a difference of 0.2 c. c. between the successive tubes.

Then add distilled water in sufficient amount to make the solution in each tube up to 10 c. c., i. e., starting with the first tube containing 6 c. c. of 1 per cent NaCl, add 4 c. c. distilled water: to the second 4.2 c. c.; to the third 4.4 c. c., etc.

When the fluids are mixed they contain a series of solutions of sodium chloride decreasing in strength by 0.02 per cent in each



successive tube. The first tube contains 0.6 per cent and the last 0.42 per cent NaCl.

Put into each tube a drop of fresh blood, shake moderately to mix thoroughly and allow the tubes to stand from 15 to 30 minutes. Observe the color of the clear fluid above the sedimented corpuscles and note the lowest percentage of NaCl in which the fluid is not tinged with red. What is the lowest percentage of sodium chloride which retains the hemoglobin in the corpuscles and prevents laking of the blood?

### **7. Spectroscopic Examination of Hemoglobin Compounds:**

Direct vision spectroscopes are used. When one looks at a bright part of the sky with a direct vision spectroscope, held so that the spectral colors extend horizontally with the red at the left, numerous vertical lines can be seen running through the spectrum (Fraunhofer's lines). These may be seen more distinctly by focussing the eye-piece and adjusting the amount of light allowed to enter the spectroscope by turning the milled ring at the prism end. The position of the line D in the orange is especially noted; also the line E in the green. The dark D line seen by sunlight is replaced by a bright line when the spectroscope is directed toward a sodium flame and this serves to fix the position of this line.

(a) *Oxyhemoglobin.* Some defibrinated blood is diluted with about ten volumes of distilled water and filtered. Some of the laked blood is then placed in an absorption cell or a test-tube and further diluted until two absorption bands can be seen. Observe that the darker one occurs in the yellow, to the right of the line D; that the one to the right is much broader but fainter and extends into the green.

(b) *Reduced hemoglobin.* Some ammonium sulphide solution is added to the solution of oxyhemoglobin and it is then heated gently to the temperature of the body. Observe that reduced hemoglobin is formed showing a single, broad band, not sharply defined, which occurs between the position of the two bands in the oxyhemoglobin spectrum.

Reduced hemoglobin may also be prepared by adding Stokes' reagent to a solution of oxyhemoglobin. Stokes' solution must be freshly prepared by dissolving two parts by weight of fer-





rous sulphate in a little water, adding three parts by weight of tartaric acid, and then adding ammonia until the reaction is alkaline.

(c) *Carbonic Oxide Hemoglobin*. Illuminating gas is allowed to bubble through a solution of oxyhemoglobin for a few minutes. The fluid changes to a bright cherry-red color due to the formation of carbon monoxide hemoglobin.

When examined with the spectroscope two absorption bands are seen in the same position as those in the spectrum of oxyhemoglobin. The bands are not identical as the band to the right (in the green) is sharper and not so broad as the one with oxyhemoglobin. The CO-hemoglobin is more stable than oxyhemoglobin and does not yield reduced hemoglobin when ammonium sulphide is added. This serves to distinguish the two compounds.

(d) *Methemoglobin*. A few drops of a potassium ferricyanide solution are added to some blood in a test-tube and the mixture is heated gently. The color is changed to a brownish or chocolate color. It is then diluted with water and examined with the spectroscope. The spectrum of methemoglobin shows a well marked band in the red and two bands, which are less distinct, between the lines D and E.

The addition of ammonium sulphide causes the band in the red to disappear and the spectrum of reduced hemoglobin is formed. This distinguishes the methemoglobin compound spectroscopically from acid hematin as the spectrum of this compound resembles that of methemoglobin very closely.

**8. The Estimation of Hemoglobin:** The normal amount of Hb. in the blood of man is 13 to 14 per cent by weight. The absolute amount is not stated in the quantitative determinations made for clinical purposes. The hemometers or hemoglobinometers used clinically indicate by colorimetric methods the percentage of the normal amount, i. e., the normal hemoglobin content of the blood is taken as 100. The calibration of some of the hemoglobinometers is too high so that 90 per cent may be regarded as the normal for healthy adults.

(a) *Tallquist's Scale*. Prick the finger and allow a drop of blood of moderate size to form. With a piece of white ab-



sorbent paper soak up the blood. After about a minute compare the color of the blood with the standard colors by passing the stain on the absorbent paper back of small openings in the color scale. The percentage is indicated by the shade which shows the same depth of color.

This method has the advantage of simplicity. The result can be determined quickly but it is not as accurate as the other methods. The colors in the scale vary by 10 per cent. This method may detect the presence of anemia but can not be depended upon to determine with accuracy the degree of anemia.

(b) *Sahl's Hemometer.* The standard color tube is a 1 per cent solution of acid hematin which is a very stable compound and keeps for years without fading. The blood taken for examination is added to dilute HCl, thus forming acid hematin so that both tubes have the same color and may be viewed in any light.

A tenth-normal sol. of HCl is placed in the graduated tube up to the mark 10. The  $\frac{N}{10}$  HCl may be prepared with sufficient accuracy by diluting 15 c. c. of strong HCl up to 1 liter with distilled water, or by taking 1.5 c. c. of strong HCl in 100 c. c. of distilled water.

A drop of blood of moderate size is obtained from the finger and the special pipette is filled a little above the 20 c. mm. mark. Any excess of blood is wiped from the end of the pipette and care should be taken to see that the lumen is filled exactly to the 20 c. mm. mark after the end of the pipette has been wiped off. The blood is then quickly expelled into the dilute acid. The lumen of the pipette is washed free from blood by drawing the acid up and expelling it several times.

Distilled water is now added gradually to the tube and mixed well with the contents until the depth of color is the same as that of the standard color tube. The percentage of the normal is expressed on the graduated tube since its capacity is 2 c. c. at the 100 mark and 20 c. mm. of blood were used. Record the result of your observation.

(c) *Gowers' Hemoglobinometer; Haldane's Modification.* The standard color tube of the apparatus originally devised by





Gowers consists of glycerin jelly tinted with carmine to the standard color of normal blood diluted to 100 volumes with water. These color tubes fade with age and Haldane has made the important improvement of substituting a sealed tube filled with a one per cent solution of carbonic oxide hemoglobin. This keeps unchanged for years.

Haldane's modification also requires the passage of illuminating gas into the mixing tube to insure complete saturation of the Hb. with CO.

The graduated tube is filled up to the 20 or 30 mark with distilled water. Blood is drawn up to the 20 c. mm. mark in the special pipette and blown into the water. Any blood adhering to the glass is washed out of the lumen of the pipette. A rubber tube attached to a gas pipe is then passed nearly to the level of the water and illuminating gas is allowed to pass for a few seconds. The end of the tube is then immediately closed with the finger and the tube is tipped back and forth at least a dozen times so that the liquid passes from one end to the other to saturate the Hb. with CO. While the tube is agitated in this way it should be held in a cloth or paper, otherwise it becomes heated from the hand and will spurt when the finger is removed.

Water is then added, drop by drop, and thoroughly mixed until the depth of color is the same as that in the standard color tube. The mark at the level of the fluid in the tube shows the percentage of Hb. in the blood. Record your observation and compare with the result by other methods

(d) *Fleischl's Hemometer.* The blood is drawn up by capillary attraction into a small capillary tube, containing 6.5 c. mm. Care should be taken that only the end of the tube touches the drop of blood and that the tube is not stuck into it, as any excess on the outside can not be wiped off easily. When the tube is exactly filled, without any blood on the outside, it is washed in a few drops of distilled water placed in one side of the cell. The capillary tube, held by the small metal handle, is first agitated in the water and then washed out and thoroughly mixed by adding more distilled water from a pipette until the chamber is filled. The other side of the cell is filled with distilled water. The two halves of the cell are



then exactly filled, without any meniscus, and covered with a cover glass, without any mixing of the fluids, to make certain that the surface is exactly level in each compartment.

Under the chamber containing the distilled water is placed a tinted glass wedge which can be moved back and forth by a thumb screw. This instrument can only be used in a dark room with yellow light, from a candle or oil lamp, reflected through the cell. The thumb screw is turned until the tints of the two sides are matched and the percentage is then read off on the scale. Several observations should be made and an average taken, working rapidly so as to avoid fatigue of the retina. Record the result.

### ENUMERATION OF CORPUSCLES.

**9. Volume Per Cent Determined by the Hematocrit:** Prick the finger and wait for a large drop of blood to collect. Fill the hematocrit tube by sucking up the blood, taking care that air bubbles do not get into the tube. When the tube is completely filled with blood, place the end of the index finger, greased with vaseline to prevent capillary attraction, over the free end of the glass tube and remove the rubber tube from the other end.

Now place the glass tube in one arm of the centrifuge with the pointed end inward. See that the spring holds it securely and that rubber washers prevent the escape of the blood. Place another empty glass tube in the other arm of the hematocrit to balance the instrument.

Set the centrifuge at full speed for two minutes. Read the volume per cent of corpuscles sedimented at the distal end of the tube. Then centrifugalize for another minute and make a second reading.

The sedimented corpuscles constitute about half the volume of blood, so that each volume per cent corresponds to 100,000 corpuscles per cubic mm. approximately. Record the result of your own observation and compare it with the count. Caution: Success by this method depends upon working rapidly and accurately. Quite uniform results may be obtained if a uniform speed of the centrifuge is used.



For clinical purposes it is inconvenient to use the hematocrit at the bedside, so that it is necessary to dilute and fix the blood. The pipette of the hemocytometer for counting leucocytes is used for diluting 10 c. mm. of blood with an equal volume of Muller's fluid or a 2 p. c. sol. of potass. bichromate. The diluted blood can then be mixed in the bulb of the pipette and blown out into a watch crystal at any time; the hematocrit tube can then be filled and the corpuscles sedimented. The percentage must be multiplied by 2 on account of the dilution of the blood.

**10. Thoma-Zeiss Hemacytometer. A. For Counting Erythrocytes or Red Corpuscles:** Select the pipette for diluting 1 c. mm. in 100 c. mm. Draw the blood up to the 0.5 c. mm. mark, taking care that exactly this amount is used after wiping the point of the pipette off; without any delay draw Hayem's fluid up to the 101 mark, making a dilution of 1 in 200; rotate the pipette by rolling it between the fingers or shaking it to mix the corpuscles uniformly.

Blow a drop or two out of the pipette and then place a small drop in the center of the cell. Cover this with a special cover glass having a plane surface.

*Caution:* The drop should not be large enough to overflow the gutter around the central cell or it will lift the cover glass up and increase the depth of the cell. If this occurs, wash the slide off and take another drop of proper size. There should not be any fluid between the cover glass and the surface on which it rests, but one should see a play of colors reflected, when the slide is held in a position to reflect the light, if the two surfaces of the glass are in close apposition.

The depth of the cell is  $\frac{1}{4}$  mm.; each side of the small squares measures  $\frac{1}{2}$  mm. The cubic content of each small square is  $\frac{1}{2} \times \frac{1}{2} \times \frac{1}{4} = \frac{1}{40}$  c. m. m. To determine the factor, this is multiplied by the degree of dilution of the blood, viz.,  $\frac{1}{200} = \frac{1}{8000}$ . The average number of corpuscles found in each small square represents  $\frac{1}{8000}$  of the number in 0.5 c. mm. of blood.

With the low power see that the corpuscles are evenly distributed; then focus the high power on the corpuscles in the squares and count the number in four large squares, prefer-





ably the four corner ones, if the corpuscles are evenly distributed in them. Average the number for each small square and multiply by 800,000. Record your results. For very accurate results from 100 to 200 small squares should be counted from two separate drops.

Clean the slide by washing with water; wash the pipette with water twice; then dry by drawing up alcohol, or alcohol and ether, and draw air through the tube, but do not blow through it. If a clot should form in the pipette, displace it with a horse hair and wash with dilute acetic acid, but never use a wire to displace a clot.

**11. B. To Count the Leucocytes:** A larger quantity of blood is used and it is diluted with 1 per cent acetic acid which should be freshly prepared. The latter lyses or dissolves the red corpuscles without changing the leucocytes, so that a dilution of 1 to 10 or 1 to 20 may be used. A weaker solution of acetic acid does not dissolve the erythrocytes completely and leaves groups of "shadows" which cause irregular distribution of the leucocytes.

Select the special pipette for making a 5 or 10 per cent dilution. Draw the blood up to the mark 0.5 and then fill the pipette to the mark 11 with freshly prepared 1 per cent acetic acid, taking care not to get any air bubbles mixed with the fluid in the pipette. After mixing the blood thoroughly by rolling the pipette between the fingers let a few drops escape, and then place a small drop on the central table of the cell as before. Take the same precautions to avoid an excess which would cause an overflow into the gutter about the central part of the cell. Apply the cover glass as before. Count the leucocytes in each large square and find the average.

In this case the number of leucocytes in each large square is  $\frac{1}{5000}$  of the number in 1 c. mm. of the blood ( $\frac{1}{5} \times \frac{1}{5} \times \frac{1}{10} = \frac{1}{250}$  c. mm.  $\times \frac{1}{20}$  dil. =  $\frac{1}{5000}$ ). Multiply the average for each large square by 5000 and record the result.

The total number of leucocytes may be counted in all of the 16 large squares and the double lines which separate them. That number should be multiplied by 200 since the cross-ruled ruled field is 1 sq. mm., the depth  $\frac{1}{10}$  mm., and the dilution 1:20.

Accurate results require counts from two drops which may be made from the same dilution.



**12. Differential Count of Leucocytes:** In determining the proportions of the different varieties of leucocytes, a thin film of blood is dried and fixed on the slide. (The method of staining these cells by Wright's stain will be given in the laboratory of clinical medicine.) At least 200 leucocytes should be counted. In all these counts the two classifications are more or less merged, since the protoplasmic granules may occur in the large mononuclear and transitional cells. The following varieties are usually spoken of in differential counts:

(a) *Small Lymphocytes.* These are small cells, usually about the size of a red blood cell. The nucleus is relatively large and stains deeply. There is little cytoplasm. These cells do not have protoplasmic granules. They seem to come from lymph glands and lymphoid tissues and have very limited amoeboid movement. They constitute from 20 to 30 per cent of the leucocytes of the blood in the adult. In the child they may be relatively much more abundant, amounting to as much as 40 or 50 per cent of the leucocytes at times.

(b) *The Large Mononuclear Leucocytes or Large Lymphocytes.* These have large nuclei and a large amount of protoplasm with or without granules. They constitute about 1 per cent of the leucocytes of normal blood.

(c) *Transitional Leucocytes.* These cells are usually considerably larger than erythrocytes; the protoplasm may contain granules having different staining properties; the nuclei are indented or lobulated and stain well. They constitute from 1 to 3 per cent of leucocytes of normal blood.

(d) *Polymorphonuclear Neutrophiles.* These cells have polymorphous nuclei with a large amount of cytoplasm. Fine granules occur in the protoplasm which take neutral stains. These are the most abundant of the different varieties, constituting from 60 to 75 per cent of the leucocytes of the healthy adult. In children the proportion of these leucocytes may be as low as 40 per cent when there is a corresponding increase in the number of lymphocytes.

(e) *Eosinophiles.* These cells may be mononuclear or polymorphonuclear. There is an abundance of protoplasm, containing coarse granules which stain deeply with eosin, or other





acid stains. These cells constitute from 1 to 3 per cent of the leucocytes.

(f) *Basophiles*. In size and shape they resemble the polymorphonuclear neutrophils. The granules are usually larger and only stain with basic dyes. With other stains they appear as vacuoles. They are very scarce in normal blood, constituting considerably less than 1 per cent. They stain well with thionin, methyl blue or dahlia.

**13. Phagocytosis:** In order to show this property of leucocytes an emulsion of water color lampblack in physiological salt sol. is injected intravenously into a dog one-half hour before the examination of the blood. A drop of blood is obtained from the ear and both fresh and fixed preparations are examined with the high power ( $\frac{1}{2}$  oil immersion lens) of the microscope.

Observe the black pigment granules in the protoplasm of the leucocytes. Observe that the polymorphonuclear leucocytes contain the greatest number of pigment granules, i. e., show the greatest phagocytic activity. These cells have relatively a large amount of protoplasm and are also most active in ameboid movements.

**14. Simple Anemia Produced by Bleeding and the Regeneration of Blood:** This experiment is performed to show (1) the changes in the blood from hemorrhage; (2) the accuracy of the methods used clinically in determining the degree of anemia, and (3) the regeneration of the blood after a considerable part (about one-fourth) of it has been removed experimentally.

A healthy dog is selected and the weight of blood in its body is calculated by taking 7.7 per cent of the body-weight. The volume of blood is then determined by dividing its weight by its specific gravity, viz., 1.055.

The ear is shaved and the skin cleansed so that several drops of blood can be obtained by pricking the ear for the following determinations of the normal blood:

- (a) Erythrocytes per c. mm.
- (b) Leucocytes per c. mm.
- (c) Hemoglobin.
- (d) Coagulation time.



To avoid the possibility of error two observations should be made in each instance and the results recorded.

The skin of the neck is shaved and disinfected with tincture of iodine and alcohol. The external jugular vein is exposed and a cannula tied in its distal end with all the precautions of asepsis. A definite quantity of the blood, amounting to 25 per cent of the total blood of the body, is then withdrawn into a graduate and the wound closed to prevent infection.

At the end of the bleeding, or immediately after it, the coagulation time and the specific gravity of the blood are again determined. These observations are repeated in 15 minutes and again one or two hours after the bleeding. At the latter time the erythrocytes and leucocytes are counted and the percentage of hemoglobin is determined.

All of these observations are repeated on each of the two days following the bleeding. The enumeration of erythrocytes and leucocytes and the determination of the hemoglobin are repeated at the end of one, two, three and four weeks after the bleeding. The determination of the coagulation time and of the specific gravity need not be repeated after the return to the normal.

Make a complete protocol of this experiment, giving a tabular statement of the different observations in separate columns and also the percentage of the normal after each observation on the erythrocytes and hemoglobin. Answer the following questions, basing your conclusions upon the results of this experiment:

1. What is the effect of hemorrhage upon the coagulability of the blood? How long does this condition last?
2. What is the effect of hemorrhage upon the specific gravity of the blood? How do you explain the alteration in the specific gravity and how long does it last?
3. Is the diminution in the number of erythrocytes proportionate to the percentage of the blood removed by bleeding? Is the erythrocyte count a reliable index of the degree of anemia?
4. Is the reduction of the normal percentage of hemoglobin in direct proportion to the degree of anemia as determined by the removal of a definite amount of blood?



5. Does a simple anemia, produced experimentally by bleeding, alter the color index of the blood, i. e., the percentage of the hemoglobin divided by the percentage of the normal number of corpuscles?

6. What alteration in the number of leucocytes follows hemorrhage and how long does it last?

7. How long does it take the blood to regenerate and return to the normal, as shown by its corpuscular and hemoglobin content, after it has been impoverished by the removal of one-fourth of the total blood of the body? How soon is the water of the blood restored as shown by the hydremia and consequent decrease in specific gravity?

**15. The Transfusion of Blood:** Successful transfusion requires (1) that the blood shall be transferred in such a manner that it does not clot while outside the body or produce intravascular clotting (thrombosis) in the recipient's vessels; (2) that there shall not be any deleterious interaction between the blood of the donor and that of the recipient.

The danger of coagulation can be avoided by using certain precautions which retard or prevent coagulation. These can be demonstrated quite as well by taking a part of an animal's blood out of the body and returning it to the vessels of the same animal, as by transfusing from one animal to another.

*Direct transfusion* consists in uniting the blood vessels in such a manner that blood may flow from one individual to another while it is kept in contact with the intima, or in interposing cannulas and a blood chamber coated throughout with a thin film of paraffin so that the foreign surface can not be wetted by the blood. The presence of a harmless, non-toxic anti-coagulant, like a sodium citrate solution, in the blood-chamber retards coagulation still further and makes it possible to transfuse two or three times longer than with paraffined apparatus alone.

*Indirect transfusion* consists in receiving blood into a 2.5 per cent of sodium citrate in the proportion of 10:1, so that the mixture contains 0.25 per cent sodium citrate. This will keep blood from clotting for days. In the amount used for transfusion it is non-toxic and does not alter the coagulability of the blood. The citrated blood may be kept for a





week or more in a sterile flask on ice and injected intravenously by introducing a needle in the vein. The indirect method has all the advantages of simplicity. It does not require any special operative skill or complicated apparatus.

The greatest danger of transfusion is *hemolysis* or the solution of the erythrocytes. The plasma of the recipient's blood may hemolyze the donor's corpuscles, or the plasma of the transfused blood of the donor may cause extensive hemolysis of the recipient's corpuscles.

Preliminary examinations should always be made by mixing the blood of the prospective donor with that of the recipient in different proportions to determine the compatibility of the two bloods before transfusion is practised. The danger of hemolysis can be avoided in that way since it does not occur *in vivo* if it is absent *in vitro*. The method of making such examinations of the blood will be given later.

While the blood of one dog may be incompatible with that of another, just as isohemolysins occur in the blood of man, for demonstration purposes a small amount of clear, citrated dog plasma may be injected into the vein of a rabbit. Extensive hemolysis occurs in a few minutes and the animal dies from cytolytic changes if a sufficient amount is injected. The blood of the dog is markedly hemolytic for the rabbit.

Observe and describe the symptoms in the rabbit.

#### 16. A. Hemolysis by Physical and Chemical Agents:

1. *Effect of Distilled Water.* Place a small drop of defibrinated blood in a small test-tube and add four or five drops of distilled water. Mix the contents of the tube well. Observe that after a short time the mixture becomes dark red in color and transparent owing to haemolysis of the red cells. Confirm this by examining a small drop of the mixture under the microscope. Hypotonic solutions act in the same way but more slowly.

3. *Effect of a Hypertonic Solution.* Repeat this experiment but use physiological salt (0.85% NaCl) solution instead of water. Observe that the mixture in the tube remains turbid and the color does not change, showing that the red cells remain in suspension and do not go into solution. Observe that under the microscope the cells show their normal shape and size.



3. *Effect of a Hypertonic Solution.* Repeat above experiment using a hypertonic salt (3% NaCl) solution. Observe that the mixture has a brighter red color, but remains opaque. This is due to the fact that the red cells are crenated and reflect more light. Confirm this by the microscope.

4. *Effect of Chloroform.* Repeat the last experiment using an isotonic solution containing chloroform instead of the solutions mentioned above. Observe that hemolysis occurs. Chloroform probably acts by dissolving the lecithin in the corpuscles. Record the result.

5. *Effect of Bile Salts.* Repeat the last experiment, using a solution of bile salts in an isotonic solution. Observe that hemolysis occurs. Bile salts produce hemolysis in the same way as chloroform and ether. Record the result.

## **B. Hemolysis by Natural and Specific Hemolysins.**

*Specific Hemolysis.* Two weeks before the observation on hemolysis is to be made, a rabbit is injected intravenously with 5 c. c. of a 10 per cent suspension of sheep's corpuscles. This injection is repeated twice at intervals of three days. On the second day before the observation the rabbit is bled and the serum allowed to separate from the blood. In like manner the serum of a normal rabbit is obtained and likewise that of a dog or cat. A sheep is also bled, the blood defibrinated and centrifugalized. The serum is removed and preserved. After washing the sheep's corpuscles three times with physiological salt solution, a 5 per cent suspension of the corpuscles in physiological salt (0.85 per cent NaCl) solution is made.

On the day of the observation half of the serum from the injected rabbit (so-called "immune serum") is heated at 55° C for 30 minutes, and half of the dog's serum is heated at 60° C for 15 minutes. This destroys the "complement" but does not destroy the "amboceptor" as shown by one of the following experiments:

We now have the following to work with: (1) Normal rabbit serum; (2) Immune rabbit serum; (3) Immune rabbit serum which has been heated to 55° C for 30 minutes; (4) Sheep's serum; (5) Washed sheep's corpuscles; (6) Normal dog's serum; (7) Dog's serum heated to 60° C for 15 minutes.



By use of the microscope and test tubes make the following observations:

1. *Agglutination.* Mix equal parts of normal rabbit's serum and washed sheep's corpuscles in a small test tube. After standing a few minutes examine a small drop under the microscope or place a small drop of each of these fluids on a slide and drop on a cover glass. Observe whether clumping or agglutination occurs and record the result.

2. *Agglutination and Hemolysis.* Mix in another tube one part of "immune rabbit serum" with three parts of washed sheep corpuscles. Note that the mixture is opaque at first and becomes transparent later due to complete hemolysis.\* Examine a drop of the mixture under the microscope from time to time, or place separate drops of the two fluids on a slide and observe the hemolysis as the fluids mix under the cover-glass. If the effects are not observed at first, repeat the observations after the mixture has stood for some time and record the result of the experiment.

3. *Effect of Heating Immune Serum.* Mix one part of immune serum heated to 55° C. with three parts of washed sheep's corpuscles. After the mixture has stood for some time, observe under the microscope as before and record the result. Agglutination may occur without hemolysis. The complement is destroyed at 55° C., while agglutinins are usually only destroyed at 60° C.

4. *Normal Serum Added to Immune Serum Heated to 55° C.* Mix equal parts of heated immune serum, washed sheep corpuscles and fresh normal rabbit or guinea pig serum. After giving time for changes to occur, examine a drop under the microscope and observe the transparency of the fluid in the tube. Record the result. Heating destroys the "complement" but not the "amboceptor" or immune body. Normal serum "reactivates" the heated serum.

5. *Natural Hemolysis.* Mix normal dog's or cat's serum with washed sheep corpuscles and observe the hemolysis. Record the result.

6. *Dog's or Cat's Serum Heated to 60° C.* Repeat the last experiment, using serum that has been heated to destroy the natural hemolysin, and record the result.





**17. Testing Donor's Blood Preliminary to Transfusion.**

**A. Rous and Turner's Method:** Use the leucocyte pipette (1-10) rinsed with 10 per cent sodium citrate solution; then draw the citrate solution up to the mark 1; the pipette is then filled rapidly to the mark 110 with blood from a large drop from the finger or ear, and *without pause* the mixture is expelled into a small, narrow test tube. This gives citrated blood with approximately 1 per cent of the citrate in the mixture.

The pipettes hold only 0.25 c. c. of fluid and that amount of blood is easily obtained. There is no objection to the use of pressure in order to get a large drop of blood rapidly. If the flow of blood ceases before the pipette can be filled, it must be expelled at once into a small test tube to prevent clotting. This mixture with citrate may then be taken up again and the pipette completely filled from a new puncture.

The pipette is rinsed with citrate solution and then with water after the blood has been collected from each individual.

**Mixing.** The mixing is done in Wright's tubes (tubes drawn out with a capillary end). Two mixtures of citrated blood are made: (a) A mixture containing nine parts of the recipient's blood to one of the donor's; (b) a mixture of equal parts of the two. In case of emergency the former will suffice, since it will show the most dangerous possibility, viz., that the blood plasma of the recipient may destroy the corpuscles of the donor.

The proportions used in mixing need only be approximate. The capillary part of Wright's tube is marked with a paraffined pencil, blood is drawn to the mark, and each column of blood separated by a small air bubble from the next one that is drawn up. To insure thorough mixing, each mixture is expelled on a slide and then drawn up in the pipette again. If the examination can not be made at once the tube may be sealed off.

A small drop of the mixture is expelled on a slide from the capillary end of the tube and a large drop of physiological salt solution is superimposed on the citrated blood without mixing. A cover glass is put on, and the preparation examined under the microscope for agglutination.

The salt solution dilutes the blood and gives a clear picture as it spreads out.

When agglutination occurs it begins in two or three minutes



and increases for a time. Observations should be made every two or three minutes. If it does not occur in fifteen minutes, the bloods may be considered compatible. In that case, the corpuscles are evenly distributed or in rouleaux as in an ordinary blood.

When agglutination occurs, the characteristic clumping may be in small masses or sometimes in large ones which can be seen microscopically. When pressure is made on the coverslip the agglutinated cells pull out in columns.

Observe and record the presence or absence of agglutination in the first mixture (a) containing one part of the donor's blood to nine parts of the recipient's blood. This represents approximately the proportion in which the two bloods will be mixed in the recipient's vessels after transfusion. Agglutination in this case indicates that the recipient's plasma agglutinates the donor's corpuscles.

Record the result in the mixture (b) which contains equal parts of the two bloods. Agglutination in these proportions, especially if it did not occur in (a), indicates that the donor's plasma acts upon the recipient's corpuscles. This is less serious as the proportion in which the two bloods should be mixed in transfusion would in all probability not result in hemolysis.

Agglutination may occur without hemolysis, but hemolysis is invariably preceded or accompanied by agglutination. It is, therefore, possible to avoid hemolysis by selecting a donor whose blood does not show any agglutination when mixed with the recipient's blood in the proportions given above.

For purposes of study by the class, the citrated blood of man, the sheep and two different dogs will be used for mixing in different proportions to determine their compatibilities.

**B. Minot's Method of Determining the Compatibility of Different Bloods:** Minot has modified and simplified the method of Rous and Turner given above. It has the advantage of diluting and mixing the bloods at once. This saves considerable time. An examination can be made by Minot's method in fifteen or twenty minutes which is about one-third of the time required for Rous and Turner's method.

The results are practically as reliable and satisfactory, al-



though neither the dilution nor the mixing is done with the same accuracy. Minot's method is as follows: Three drops of a 1.5 per cent solution of sodium citrate in 0.9 per cent NaCl sol. are placed in each of two watch crystals or small test-tubes. In the first one, nine drops of the recipient's blood are received directly into the citrate solution and mixed with one drop of the donor's blood. In the second, nine drops of the donor's blood are mixed with one of the recipient's in the same way.

The two mixtures are agitated or stirred to insure thorough mixing, and allowed to stand for fifteen minutes. At the end of that time a drop is placed on a slide and examined under the microscope. Agglutination in the first mixture indicates that the recipient's plasma is incompatible with the donor's corpuscles and would possibly hemolyze them as explained before. Agglutination in the second mixture indicates that the donor's plasma is incompatible with the recipient's corpuscles.

The absence of agglutination shows that the two bloods are compatible and that hemolysis will not occur. Agglutination may occur without hemolysis, but hemolysis does not occur without agglutination.

The amount of blood required for this test can easily be obtained by pricking the finger and allowing the blood to drop directly into the citrate solution. The drops may not be of the same size but that does not matter as the mixture is only approximate and is sufficiently accurate for clinical purposes.

**18. Blood Proteins:** (a) *Fibrinogen*. Some citrated or oxalated plasma is obtained by sedimenting the corpuscles with the centrifuge. The fibrinogen is precipitated by half-saturation with sodium chloride. Other globulins require full saturation with sodium chloride for precipitation.

(b) *Serum Globulin*. After filtering off the precipitated fibrinogen in the last experiment, an equal volume of a saturated solution of ammonium sulphate is added to the filtrate. Observe that serum globulin is precipitated by half saturation with ammonium sulphate.

(c) *Serum Albumin*. The filtrate from the last experiment is completely saturated with ammonium sulphate by adding the crystals until no more can be dissolved. Observe that serum albumin is precipitated.





**19. Coagulation of Blood:** Coagulation of the blood may be prevented by the following methods:

1. Receiving the blood into a vessel surrounded by a freezing mixture of ice and salt.

2. Receiving blood into a saturated solution of  $MgSO_4$  in the proportion of three of the former to one of the latter.

3. Receiving the blood into a solution of an oxalate to form a mixture of 0.2 per cent or more of the oxalate.

4. A mixture of 0.25 per cent or more of sodium citrate.

5. A mixture of at least 0.3 per cent of sodium fluoride.

6. Hirudin in the proportion of 0.001 gram. to 8 c. c. of blood. This prevents extravascular clotting and also destroys the coagulability of the blood when injected into an animal without having any toxic effect. Hirudin is a proteose body prepared from the heads of leeches and acts as an antithrombin.

7. The intravenous injection of commercial peptone, in the proportion of 0.3 to 0.5 gram. per kilogram of body weight, usually, but not invariably, destroys the coagulability of the blood. Sometimes it fails to lessen the coagulability. Peptone does not have any effect on extravascular clotting when shed blood is received into a peptone solution.

*Magnesium Sulphate Plasma.* Dilute a fresh mixture of 3 parts of blood in 1 part of sat. sol.  $MgSO_4$  with 4, 8, 10 and 12 parts of distilled water. Observe that dilution with four volumes of water is not sufficient to cause clotting; that clotting occurs when fresh "salted plasma" is diluted with 8 or 10 parts of water. If the blood has been standing on ice for 24 hours or more, a greater dilution may be required. If the corpuscles are centrifugalized from such blood the clear plasma may not clot on dilution or even on the addition of tissue extracts.

Apparently the  $MgSO_4$  interferes with the interaction of the fibrin factors, and if the mixture is allowed to stand for 24 hours or more, some essential factor is precipitated.

*Oxalated Blood.* A 0.5 per cent oxalated blood is obtained by receiving 3 parts of blood into 1 part of 2 per cent ammonium oxalate, and this mixture is used for the following experiments:

(a) Observe that dilution, even with 15 or 20 parts of distilled water, does not cause clotting.



(b) The addition of 6-8 drops of 2 per cent  $\text{CaCl}_2$  to 5 c. c. of oxalated blood is not sufficient to recalcify the blood, but 10 or more drops cause clotting.

(c) Tissue extracts, prepared by grinding up liver and muscle in physiological salt solution after these tissues have been washed free from blood, do not cause clotting.

(d) Serum causes clotting when added in sufficient amounts (2-5 c. c. of serum to 5 c. c. oxalated blood). The comparison between the serum and tissue extracts can best be shown by partially, but not completely, recalcifying the blood by adding 3 or 4 drops of 2 per cent  $\text{CaCl}_2$ . It is clear from these experiments that soluble oxalates prevent coagulation solely by removing the calcium in the formation of an insoluble oxalate. The dilution of blood or the addition of tissue extracts can not cause clotting unless the blood is recalcified.

*Citrated Blood.* While 0.25 per cent of sodium citrate will prevent blood from clotting, a 0.5 per cent mixture is used for the following experiments:

(a) Dilution with distilled water does not cause clotting. This indicates that the citrate does not prevent cellular disintegration or any interaction between the fibrin factors.

(b) The addition of 2 drops of 0.25 per cent sol.  $\text{CaCl}_2$  to 5 c. c. of 0.5 per cent citrated blood does not cause clotting, but the addition of 4 or more drops causes a clot to form. From this it is clear that citrated blood is much more easily recalcified than the oxalated blood. In the latter an insoluble precipitate of calcium oxalate is formed, while the citrate forms a double salt with the calcium of the blood, viz., calcium sodium citrate which is not insoluble but non-ionizable.

(c) Tissue extracts do not cause citrated blood to clot unless enough of the extract is added to recalcify the blood.

(d) Blood serum causes coagulation by the excess of thrombin contained in it.

*Fluoride Blood.* A 0.6 per cent sodium fluoride mixture is obtained for the following experiments by receiving 3 parts of blood directly from the blood vessel into 1 part of a 2.4 per cent sodium fluoride solution.



(a) Dilution with water does not cause clotting.

(b) The addition of an excess of  $\text{CaCl}_2$  (10 drops of 2 per cent  $\text{CaCl}_2$  to 5 c. c. fluoride blood) may cause clotting. The addition of 5-6 drops of 2 per cent  $\text{CaCl}_2$  may be sufficient to recalcify 5 c. c. of blood by combining with all the excess of fluoride to form an insoluble calcium fluoride, but clotting does not occur.

(c) The addition of tissue extracts does not cause clotting.

(d) The addition of blood serum in sufficient amount (2-5 c. c. serum to 5 c. c. blood) causes clotting.

(e) Recalcified fluoride plasma is prepared by adding 6 drops of 2 per cent  $\text{CaCl}_2$  to 10 c. c. of fluoride blood. The above tests are then repeated. Observe (1) that dilution with water now causes clotting; (2) that tissue extracts now cause clotting as well as blood serum.

These experiments indicate that sodium fluoride prevents coagulation partly by decalcifying the blood, and in addition, by preventing cellular disintegration and the liberation of a thromboplastic substance (thrombokinase).

*Peptone Plasma.* The injection of commercial peptone does not always destroy the coagulability of blood, but if peptone plasma can be obtained the following experiments may be performed:

(a) Dilute with 4, 8, and 12 volumes of distilled water and record the result. Dilution usually causes peptone plasma to clot, showing that the interaction between the precursors of fibrin is prevented in some way.

(b) The addition of  $\text{CaCl}_2$  alone does not cause it to clot.

(c) The addition of tissue extracts, in the same proportions that were used in the previous experiments causes a clot. It is clear from this experiment that the formation of thrombin has been prevented in some way. The thromboplastic substance (thrombokinase) appears to be inactive or unavailable for some reason.

(d) To 5 c.c. of peptone plasma add blood serum in increasing quantities, starting with 1 c.c. and increasing 1 c.c. at a time until coagulation occurs. Record the result. Observe that much more of the serum is required than of the tissue extracts.





The reverse is true of fluoride and citrated blood. The fact that an excessive amount of serum, which contains thrombin, has to be added indicates that peptone plasma probably contains an antithrombin.



## DIGESTION.

**20. Comparative Anatomy of the Alimentary Canal:** Copy the table showing the proportion of the length of the body to the length of the alimentary canal in the cat, dog, horse, pig, ox, sheep and in man.

Copy the tabular statement showing the relative capacities of the stomach, small intestine and large intestine in the dog, horse, sheep and pig.

Copy the tabular statement showing the length of the small intestine and the length of the large intestine in the cat, dog, rabbit, and pig; also in man at birth and in an adult man. Examine the anatomical specimens of the alimentary tract. Examine particularly the stomach, the pyloric sphincter, the wall of the antrum, position of the transverse band, fundus, etc.

From these observations briefly state your conclusions concerning (1) the relation of the nature and amount of food consumed to the length and capacity of the alimentary canal; (2) to the length of the small and large intestine, respectively; (3) state in a broad way the function of the stomach, small and large intestine, respectively.

Examine the skulls of rodents, carnivora, herbivora and omnivora showing the articulation of the mandible with the skull; also the kinds and arrangement of the teeth. Examine the temporary and permanent teeth in man.

**21. Influence of the Mechanical Comminution of Food on the Chemical Changes of Digestion:** 1. *Artificial Digestion.* Some raw egg white is stained deeply with ammonia carmine and then slowly coagulated by heat so that the stain is fixed in the coagulum and can only go in solution in an artificial peptic digest as proteolytic digestion occurs.

The coagulated egg white is divided into two equal portions by weight. One part (A) is cut up into large cubes; the other part (B) is finely ground up by passing it through a meat-chopper. Each part is then put in a beaker containing the same amount of pepsin and 0.4 per cent HCl. Both beakers are kept in the incubator at 38° C. for 4 or 5 hours. Contrast the amount of digestion in the two beakers by comparing the



depth of coloring with the carmine that has passed in solution and record the result.

2. *Natural Digestion.* Four dogs are fasted long enough to make certain that their stomachs are empty. The first two, A and B, are each given 100 grams of lean meat cut in large cubes so that the food has to be masticated preparatory to deglutition.

Two other dogs, C and D, are each given 100 grams of lean meat that has been finely ground up by passing it through the meat-chopper.

At the end of three hours, a hypodermic injection of 0.2 of a grain of apomorphine is given to dogs A and C, and the vomited meat, which is promptly and completely expelled, is collected in a pan.

At the end of four, or four and one-half hours, the meat is recovered from the stomachs of the dogs B and D in the same way.

Contrast the amount of food that remains undigested in each case after three and after four hours. Write a brief description of the appearance of the meat and state how thorough comminution favors digestion.

**22. The Chemical Changes of Gastric Digestion:** The meat recovered after 3 and 4 hours of natural gastric digestion in the preceding experiment is suspended in water and examined for the different stages of proteolysis as follows:

- (a) Acid albumin (neutralization).
- (b) Primary proteoses (half saturation with  $(\text{NH}_4)_2\text{SO}_4$ ).
- (c) Secondary proteoses (complete saturation with  $(\text{NH}_4)_2\text{SO}_4$ ).
- (d) Peptones (Biuret test after removing proteoses).

Record the result. Observe that all occur.

In the same way some of the natural digest of 3 and 4 hours, respectively is titrated against  $\frac{\text{N}}{10}$  NaOH and the total acidity, free and combined HCl are determined. Record the result.

**23. Conditions Affecting Peptic Digestion:** The same amount of fibrin stained with ammonia carmine is placed in each of the following test-tubes and the artificial digests are kept in the incubator for several hours:





- (a) Pepsin in 0.4 per cent HCl (control).
- (b) Pepsin in neutral solution.
- (c) 0.4 HCl alone without pepsin.
- (d) Pepsin + 0.4 p. c. HCl boiled.
- (e) Pepsin + lactic acid (equivalent to 0.4 per cent HCl).
- (f) Pepsin + 0.1 per cent HCl (hypoacidity).

Observe the depth of color from the dissolved carmine and record the results.

Mett's tubes may be used to show that the amount of enzyme influences the rapidity of digestion.

**24. Conditions Affecting the Action of Rennin:** Test tubes are prepared having the same amounts of rennin and milk, as follows:

- (a) Rennin + milk (control).
- (b) Boiled rennin + milk; enzyme destroyed.
- (c) Neutralized rennin + milk.
- (d) Rennin + milk diluted with equal volume of water.
- (e) Rennin + human milk (very fine curd).
- (f) Rennin + citrated milk. Clots on addition of  $\text{CaCl}_2$ .

The digest may be boiled before adding  $\text{CaCl}_2$ .

**25. Conditions Affecting the Digestion of Curdled Milk:**

Small beakers are prepared each having the same amount of milk (100 c. c.) plus pepsin in 0.4 p. c. HCl.

- (a) Milk plus pepsin in HCl (control).
- (b) Milk diluted with an equal part of water.
- (c) Milk diluted with an equal part of starch water.
- (d) Milk mixed with bread crumbs.
- (e) Milk mixed with cracker crumbs.

Observe and record the result in each case. What is the advantage of starch water over plain water as a diluent for milk in promoting gastric digestion? How do bread or cracker crumbs favor the digestion of milk?

**26. Conditions Affecting the Acidity of the Gastric Juice:**

Compare the amount of total, free and combined HCl in the stomach content of several different dogs as determined by titration after a test-meal of cracker crumbs suspended in water and removed by a stomach tube at the end of one hour.

Contrast the above control in each case with the determina-



tion of total, free and combined HCl after feeding (a) with milk; (b) with egg white; (c) with meat after 2 and 4 hours, respectively. How do foods rich in proteins compare with a test-meal of crackers or bread crumbs in exciting the flow of HCl?

How does meat compare with egg-white in stimulating the secretion of HCl when relatively the same amount of protein is given, diluted to the same volume with water?

How does milk compare with the test meal of cracker crumbs in water?

**27. The Influence of Meat Extractives on the Secretion of Gastric Juice:** Two animals (dogs or cats) may be anesthetized and have the cardiac and pyloric orifices of the stomach tied off after a certain amount (from 75 to 200 c.c.) of water has been introduced into the empty stomach of one, and the same amount of bouillon into the empty stomach of the other. The animals may be kept lightly under ether and at the end of one hour the fluid is collected from each animal, measured and titrated against  $\frac{N}{10}$  NaOH. Record the result. Observe that very little (not more than 5 per cent) of the water is absorbed and it practically does not cause any secretion of HCl; that the amount of fluid is increased in the stomach containing bouillon and the acidity is three or four times greater than that containing water.

The influence of creatin as a gastric secretagogue may also be shown by giving a test-meal of cracker crumbs suspended in bouillon and comparing it with the acidity found in a previous test-meal of cracker crumbs and water in the same dog. The stomach contents removed by a stomach tube in this way can only be used for comparisons in acidity. The amount of secretion can not be determined as some fluid escapes through the pylorus.

**28. Psychic Secretion of Gastric Juice:** Two cats or dogs are starved for one or two days until they show evidence of hunger. One of them is then placed in a cage with a false floor made of wire mesh. Meat or fish is placed beneath the wire mesh so that the animal can see and smell the food but can not eat it. After several hours of excitement caused



in this way, both animals are placed under an anesthetic and a cannula is introduced into the stomach of each through the pyloric splineter from the duodenum. The fluid contained in each stomach is collected, measured and titrated. Record the result.

If the sight and odor of food caused much excitement, there is a decided increase in the quantity and in the acidity of the fluid in the stomach of that animal as compared with the content of the other animal starved for the same time but not excited by food.

**29. Mechanical Stimulation of the Gastric Mucosa:** When inert and insoluble substances, such as short pieces of a wax taper, are introduced into the stomach of a dog with a definite amount of water, observe that the fluid drawn off from the stomach at the end of a half-hour or one hour shows very little increase in total acidity and no free HCl. Record the result and contrast with the secretion caused by cerebral excitement; also with that caused by the secretagogue creatin in the preceding experiment. Compare the result of mechanical stimulation in this experiment with the result obtained in the next one.

**30. The Influence of Secretory Nerve Fibers on the Gastric Glands:** One vagus nerve, preferably the right, may be cut, with the precautions of asepsis, three days before this experiment is performed. This allows the cardio-inhibitory fibers to degenerate but does not interfere with respiration. The secretory nerve fibers to the gastric tubules do not degenerate as rapidly as the cardio-inhibitory fibers and therefore they may be stimulated by stimulating the peripheral end of the nerve with a tetanizing current three or four days after cutting it. In this way any disturbance of the circulation is avoided.

Or, both vagi may be stimulated by single induction shocks at intervals of one second without preliminary section of the nerve. These are sufficient to excite the gastric glands to activity without lowering the blood-pressure.

A gastric fistula is produced and the two openings tied off in a fasting dog; the wound is closed, and the stomach is washed out with warm water. Mechanical stimulation may be applied for 15 minutes by introducing a feather or several wax tapers. Any





secretion caused by the mechanical stimulus is usually alkaline from mucus.

After again washing out the stomach, the animal is placed in a special holder so that the gastric juice drains from the cannula as it is formed. The secretory fibers of the vagus are stimulated by one of the methods mentioned above for twenty minutes. Observe the long latent period. Secretion does not begin until the nerves have been stimulated for 6 or 7 minutes. After secretion begins, the gastric juice is collected for 5 minute periods. Make a protocol of the experiment showing the latent period and the amount secreted for the different periods.

If secretion does not occur, on account of the anesthesia and surgical shock, a small dose of pilocarpine may be given intravenously and the stimulation applied as before. If this succeeds an injection of atropine may subsequently be made and the stimulation applied as before. Pilocarpine stimulates the secretory fibers while atropine paralyzes them.

The gastric juice collected in this experiment is titrated for total acidity and free HCl. The digestive enzymes may be shown by adding some of it to milk and another part to fibrin, keeping the test-tubes at the bodily temperature.

Copy a protocol of the secretion obtained in various ways from a dog with a fistula of the stomach by Pawlow's method.

**31. The Digestion of Living Tissues; Auto-digestion:** Five frogs have their brains pithed and are given enough curare to produce motor paralysis. They are then suspended so that one leg of one is kept in a beaker containing 0.2 per cent HCl; another in a beaker containing pepsin and 0.2 per cent HCl; the third in a neutral solution of trypsin; the fourth in a solution of trypsin in 1 per cent  $\text{Na}_2\text{CO}_3$  and the fifth in 1 per cent  $\text{Na}_2\text{CO}_3$ .

All of the beakers are kept in a water bath at  $37^\circ\text{C}$  for 3 or 4 hours. Observe that pepsin and HCl cause complete digestion of all the soft tissues with which the digest comes in contact; that the acid or the alkali alone, or the trypsin cause maceration of the skin, but not complete solution of the tissues. Record the result.

The bladders of dogs filled with pepsin and HCl show the



same digestion, even to the extent of perforation. The animals were narcotized but the circulation was normal. Can the circulation of the alkaline blood and lymph explain why the living stomach does not digest itself, as the wall of the dead stomach is digested when removed during active digestion and kept at the bodily temperature for a few hours?

Antiferments may be shown by adding a small amount of a glycerine extract of tape-worms to an artificial digest of fibrin in pepsin-HCl and also to an artificial digest of fibrin in trypsin and  $\text{Na}_2\text{CO}_3$ . These are compared with control digests and the influence of the antiferment in restraining proteolytic digestion can be seen. This explains how intestinal parasites and the intestinal mucosa escape digestion. The bearing of auto-digestion upon the pathogenesis of gastric and duodenal ulcers is obvious.

### **32. The Action of Secretin on the Flow of Pancreatic Juice:**

A solution of secretin is extracted from the intestinal mucosa as follows: The mucous membrane from the duodenum and upper 3 or 4 feet of the jejunum is scraped off and chopped up finely. It is then mixed with washed sand and ground up in a mortar with 150 c.c. of 0.2 per cent HCl. It is allowed to stand until a short time before the experiment, when it is filtered through gauze. It is then carefully neutralized by adding NaOH. After bringing it to the neutral point a few drops of dilute acetic acid are added to give it a faintly acid reaction.

An extract from the lower end of the ileum is prepared in the same way.

A dog is anesthetized, the abdomen opened and cannulas are introduced into the common bile duct and into one of the pancreatic ducts. The cystic duct is ligated and the number of drops falling from each of the cannulas is counted for two periods of two minutes each.

An intravenous injection of 10 c.c. of the extract from the ileum is then made and the number of drops counted again. Record the result.

The same amount of the extract of the duodenum and jejunum is then injected intravenously. The number of drops is again counted and recorded.



Is secretin contained in the mucous membrane of the lower end of the ileum as it is in the upper part?

Is the flow of bile stimulated to the same extent as the flow of pancreatic juice?

How long does the flow continue? An injection of dilute HCl (0.2 per cent) may be made into the lumen of the duodenum when the flow of pancreatic juice returns to the normal after the intravenous injection of secretion. The number of drops is counted again and recorded.

**33. Pancreatic Digestion:** Some fresh pancreatic juice is collected from a cannula tied in the pancreatic duct of another dog. A fistula of this kind may be used to collect the pancreatic juice before it enters the intestine. Describe its appearance and give its reaction.

*A. Action on Starches.*

1. Add 5 or 10 drops to some 0.7 per cent solution of boiled starch at the temperature of the body and test for sugar every 15 or 20 seconds until it appears. Record the result. Boil with Barford's reagent and observe that dextrose is not formed.

2. Repeat this experiment using a suspension of raw starch that has been ground up in a mortar, or use raw potato and compare with cooked potato. Record the result.

3. Repeat the experiments using diastase, a commercial extract of pancreas, instead of the pancreatic juice.

4. Repeat 1 and 2 using saliva in the same amount, instead of pancreatic juice, and compare the time required for the appearance of maltose.

5. Add 10 drops of pancreatic juice to each of two test-tubes, A and B. To the tube B add 10 drops of a 3 per cent solution of bile salts. Now add a lukewarm solution of boiled starch to each and, after waiting 15 to 30 seconds, add 1 or 2 drops of a weak iodine sol. (Lugol's sol.). Invert the tubes repeatedly and observe the rapid change from starch to erythrodextrin and achroodextrin in B as compared with A. This is shown by the change in color from blue to violet, then to red and finally the disappearance of all color with the formation of the final products of starch digestion.





What is the influence of bile on the pancreatic digestion of starches?

Are the bile salts necessary to activate the amylase or is the pancreatic amylase capable of digesting starches before it comes in contact with bile?

How do co-ferments differ from activators?

**B. Action on Fats.** Some milk is boiled to kill the acid-producing bacteria in it. A few drops of phenolphthalein are added and enough  $\frac{N}{10}$  NaOH is then cautiously added to give it a distinct and permanent pink color. The degree of fat digestion will be indicated by the amount of fatty acids liberated as determined by titration with  $\frac{N}{10}$  NaOH.

1. Take the same amount of lukewarm, alkaline milk containing phenolphthalein as prepared above in each of two test-tubes, A and B. To A add 10 drops of fresh pancreatic juice; to B add 10 drops of pancreatic juice that has been boiled in a little water in another tube. Place both tubes in a water-bath at 37° C for 3 or 4 minutes. Observe the rapid disappearance of the pink color in A. Then add  $\frac{N}{10}$  NaOH from a burette or graduated pipette to each until a permanent pink color is again established. Record and explain the results.

2. Take three test-tubes labeled A, B and C. To A and B add 10 drops of fresh pancreatic juice; to B and C add 10 drops of a 3 per cent sol. of bile-salts. Add the same amount of alkaline milk containing phenolphthalein, as prepared above, to each one and place all of the test-tubes in a water bath at 37° C for 4 or 5 minutes. At the end of that time boil each tube to stop the enzyme action. Now titrate  $\frac{N}{10}$  NaOH into each one until a permanent pink color is produced and record the result.

Can the pancreatic lipase, steapsin, act without bile-salts?

What is the influence of bile-salts upon the pancreatic digestion of fats?

Can bile-salts alone cause the hydrolytic cleavage of neutral fats?

Do the bile-salts act as activators or as co-ferments?

3. The influence of emulsification in promoting the digestion of fats by the pancreatic lipase may be shown by the following experiment: The same amount (3 c.c.) of neutral cottonseed



oil is taken in each of two test-tubes, A and B. Some distilled water (about 5 or 6 c.c.) is added to A and the same amount of a solution of ivory soap is added to B. Both tubes are shaken well. An emulsion is formed in B while the fat separates at the top of A. 1 c.c. of fresh pancreatic juice is then added to each and the tubes are placed in a beaker of lukewarm water for 10 minutes. At the end of that time the tubes are boiled to stop further digestion. A few drops of phenolphthalein are added and  $\frac{N}{10}$  NaOH sol. is titrated into each. Record the result.

How does emulsification aid fat digestion by steapsin?

4. Repeat the experiments 1, 2 and 3, using a fresh solution of holadin instead of the pancreatic juice. Holadin is a commercial extract of pancreas containing the pancreatic lipase. Record the result and compare with the results obtained in 1 and 2.

C. *Action on Proteins.* To each of three test-tubes, A, B and C, add 15 drops of fresh pancreatic juice obtained from a cannula tied in the pancreatic duct. To B add some scrapings of the intestinal mucosa, and to C add the same after it has been boiled. Fill each tube with water and add the same amount of fibrin stained with carmine to each tube. Keep all of the tubes in the incubator at 38° C for several hours and then compare the degree of digestion by the depth of the color from carmine in solution in each tube; also by the amount of fibrin that remains undigested. Explain the difference.

Is the proteolytic enzyme secreted by the pancreas in active form, or is it activated by something contained in the intestinal mucosa?

What is the activator of trypsinogen and what is the nature of its action?

**34. Conditions Influencing Tryptic Digestion:** Seven test-tubes are labeled A, B, C, D, E, F and G. The same amount (1 c.c.) of an extract of pancreas or a solution of pancreatin is added to each one except G. A is boiled; B and C are neutralized using litmus as an indicator; 1 c.c. of a solution of bile-salts is added to C; D is made faintly acid with dilute lactic acid; E is made acid with 0.2 per cent HCl. The tubes A, B, C, D and E



are then filled with distilled water. The tubes F and G are filled with 1 per cent  $\text{Na}_2\text{CO}_3$ . The same amount of fibrin stained with carmine is then added to each tube and the tubes are kept in the incubator for 3 or 4 hours. At the end of that time the tubes are inverted and the depth of color from carmine in solution is compared to determine the degree of digestion in them. Record the results in answering the following questions:

1. Does boiling destroy trypsin (tube A)?
  2. Can trypsin act in a neutral medium (B)?
  3. Is the action of trypsin intensified by bile-salts as much as the other pancreatic enzymes (C)?
  4. Can trypsin act in the presence of an organic acid (D)?
  5. Can trypsin act in the presence of dilute  $\text{HCl}$  (tube E)?
- Trypsin is destroyed by *free* but not by combined  $\text{HCl}$ .
6. Is the digestion in an alkaline medium equal to that of the pancreatic juice (tube F) more active than that in a neutral medium (tube B)? Compare tubes F and G and allow for the solution of the carmine by the  $\text{Na}_2\text{CO}_3$  alone as seen in tube G.

**35. The Peptonization of Milk:** The same amount of milk (100 c.c.) is taken in each of six beakers A, B, C, D, E and F. To A and B add pepsin dissolved in 0.2 per cent  $\text{HCl}$ ; to C and D add pancreatin or extract of pancreas and enough sodium bicarbonate to give an alkaline reaction; to E and F add peptogenic milk powder.

Place all six beakers in the incubator at  $37^\circ\text{C}$ . At the end of 15 minutes take A, C, and E out and boil to stop further digestion. At the end of 30 minutes remove B, D and F and boil.

Observe the undigested curd in A and B. Why is it impracticable to peptonize milk with pepsin and  $\text{HCl}$ ?

Apply the following test to C and E to determine the amount of caseinogen which remains undigested, comparing the result in each case with a control test in which the same amount of undigested milk is treated in exactly the same way.

1. Dilute some of the milk in a test tube with four parts of warm water and add dilute acetic acid from a pipette until the caseinogen is precipitated in curds. The mixture is kept warm by passing it through the flame from time to time while adding the acid.





Repeat this test with some of the milk from the beakers D and F, using the same degree of dilution and cautiously add dilute acetic acid until a fine precipitate forms. An excess of acid should be avoided as it re-dissolves the precipitate.

Contrast the *amount* and particularly the *character* of the precipitate in C and E with that in the control. Observe the further change in D and F.

What change takes place in milk after peptonization for 10 or 15 minutes with the active proteolytic enzyme extracted from the pancreas?

How does this change influence the digestibility of milk when it is subsequently taken into the stomach?

What is the action of rennin on peptonized milk?

2. In which ones of the six beakers has a bitter taste developed and what is the cause of it? How can this unpleasant taste be prevented when milk is properly peptonized?

3. Apply the biuret test and observe the pink or rose color of proteose in the peptonized milk as compared with the bluish violet color of undigested milk. The same amount of milk is taken from the beakers C, D, E and F in each of four test tubes and an equal amount of undigested milk is taken to a fifth tube. An excess of 15 per cent NaOH is then added. A few drops of 1 per cent  $\text{CuSO}_4$  are then added until the same depth of color is obtained in each tube.

**36. Bile. Hay's Test for Bile Salts.** Take the same amount of water in each of three test tubes, A, B and C. Add 10 or 15 drops of bile to A and the same amount of a sol. of bile salts to B. Keep C as a control. Sprinkle a few particles of powdered sulphur on the surface of each. Observe that the sulphur quickly sinks to the bottom of A and B but floats on the surface of the water in C. Bile salts lower the surface tension of fluids in which they are dissolved and for this reason the sulphur sinks to the bottom. It can not be wetted in the tube C. This is a very delicate test and it can detect 1 part of bile salts in 100,000 of water. It is a very satisfactory test for detecting bile salts in the urine when the outflow of bile into the intestine is obstructed with consequent absorption into the blood.



**37. The Gmelin-Heintz Test for Bile Pigments:** A few drops of bile, or a weak solution of bile, are placed on a white plate and allowed to come in contact with impure  $\text{HNO}_3$  containing  $\text{HNO}_2$ . Observe the play of colors at the line of contact from the oxidation of the bile pigments: green, blue, violet, red and yellow.

**38. Interaction between the Bile and the Chyme:** Take four test-tubes A, B, C and D. Add 5 c.c. of 0.2 per cent  $\text{HCl}$  to A and B and the same amount of a filtered peptic digest to C and D. To A and C add a solution of bile salts until a precipitate forms; to B and D add fresh bile. Observe a slight precipitate of bile salts in A and a heavier precipitate of mucin and bile salts in B. In C the products of digestion are precipitated; in D the products of digestion are precipitated, in addition to the action of the acid on the bile, giving the heaviest precipitate of all. When an excess of bile or of the solution containing bile salts is added, most of the precipitated products of digestion are re-dissolved.

**39. Solvent Action of Bile on Insoluble Soaps:** Take 5 c. c. of a soap solution in each of two test-tubes, A and B. Add a drop or two of a  $\text{CaCl}_2$  sol. to each and observe the formation of an insoluble calcium soap. To A add a solution of bile salts and to B add the same amount of distilled water. Shake the tubes and observe that the insoluble calcium soap is dissolved by the bile salts in the tube A.

**40. Solvent Action of Bile on Fats:** Moisten two filter papers with a 5 per cent solution of bile salts on funnels A and B. Moisten two other filter papers with water on funnels C and D. Pour neutral cottonseed oil on the filter papers in the funnels A and C and rancid fish oil in B and D. Observe that the solvent action of bile on neutral fats allows some of the oil to pass through the filter paper in A, while the greater solvent acid for fatty acids makes it possible for a larger amount of the rancid fish oil to pass through the filter B in the same time.

None of the oil can pass through the filters C and D while they are wet with water.

**41. Intestinal Digestion of Proteoses by Erepsin:** A 0.5



per cent of commercial "peptone" is prepared and the same amount is taken in each of three beakers, A, B and C. To A and B the same amount of the scrapings from the intestinal mucosa is added. B is boiled to destroy the enzyme.

A and B are placed in the incubator at 37°C for several hours. At the end of that time the following tests are applied to some of the digest from A and B, respectively, comparing each one with a control test from the original peptone solution in C:

1. Apply the biuret test. Observe that proteoses disappear from A, containing active erepsin, but remain in B.

2. Hoffman's test for tyrosin. Add Millon's reagent and boil. Observe the deep red color in the solution in A as compared with the light dull red color of the precipitate in B and C. The latter is due to the proteins while the former is due to tyrosin.

3. Apply the tryptophan test by adding bromine water to some of each of the solutions. This is only obtained with the digest A.

If these reactions do not show after the digest has been in the incubator four hours, the digestion should be continued for 24 hours and the tests repeated.

**42. Inverting Enzymes of the Intestinal Mucous Membrane:** Scrapings or shreds of the intestinal mucosa are put in 1 per cent solutions of the following: (a) Sucrose; (b) maltose; (c) lactose and (d) dextrin. A portion of each digest is then boiled to destroy the enzymes, and all of the digests, boiled and unboiled, are placed in the incubator for several hours.

A portion of each digest is then boiled with Barfold's solution and a positive reaction shows the formation of dextrose by the inversion of the di-saccharides.

**43. Movements of the Stomach and Intestines:** A cat is fed with finely chopped meat mixed with boiled corn meal, four hours before the experiment. The cat is anesthetized through a tube tied in the trachea and is fastened in a special holder so that it can be completely immersed in Ringer's solution. The Ringer's solution is kept at the temperature of the body and



prevents contact of the viscera with the air when the abdomen is opened.

A long median incision is made in the abdominal wall and the stomach, small intestine and large intestine exposed to view while these viscera are kept submerged beneath the Ringer's solution. If the normal movements do not occur they may be set up by stimulating the vagi in the neck with induction shocks at intervals of one second, the stimuli being interrupted from time to time.

Observe where the movements begin in the wall of the stomach and the way in which they pass to the pylorus. Observe particularly the change at the transverse band and in the wall of the antrum; also the succession of contraction waves.

Observe the rhythmic and the peristaltic waves in the wall of the small intestine and the distance the latter travel.

Observe the reversed peristalsis or antiperistaltic waves in the ascending colon.

If these movements only occur from electrical stimulation of the accelerator fibers in the vagi, observe how they subside when this artificial stimulation is discontinued from time to time.

Write an account of the observations made in this experiment.





## RESPIRATION.

### CHANGES IN THE AIR FROM RESPIRATION.

**44. Composition of the Atmosphere:** (a) *Percentage of Oxygen:* A definite amount (100 c.c.) of the air of the laboratory is measured in a Hempel burette. It is then forced into a Hempel gas pipette over a concentrated solution of pyrogallie acid and caustic potash. This absorbs the oxygen and the  $\text{CO}_2$ , but the amount of the latter is so slight in this case that it may be disregarded.

The air is then forced back into the burette, the pressure equalized, and the volume noted. The diminution in volume in 100 c. c. of air indicates the volume per cent of oxygen in the air of the room. Record the result.

(b) *Percentage of  $\text{CO}_2$  in the Atmosphere:* Low percentages of  $\text{CO}_2$  can not be determined by the slight reduction in a small volume of air after absorption by a caustic alkali in a gas pipette. The most accurate method is to absorb the  $\text{CO}_2$  from a large volume of air by shaking with lime water, or baryta water, of definite strength, and determine the consequent reduction in alkalinity by titrating against a weak standard acid of definite strength (Pettenkofer's method).

A convenient method is that suggested by Dr. Angus Smith, which has been standardized by comparison with Pettenkofer's method. It depends upon the amount of air required to produce turbidity in lime water. The larger the amount of air required, the lower the percentage of  $\text{CO}_2$ ; the smaller the volume of air required to produce turbidity, the higher the percentage of  $\text{CO}_2$ .

Bottles of different sizes are unstoppered in a room so that the air of the room may mix by diffusion with the air in the bottles; into each bottle a small amount (15 c. c.) of freshly filtered lime water is placed; the bottles are then stoppered and shaken for a few minutes. Distinct turbidity of the lime water in any but the largest bottles indicates an excess of  $\text{CO}_2$  above the normal (0.03 to 0.04 per cent). Turbidity in the different bottles indicates the following percentages:



|                  |   |                              |
|------------------|---|------------------------------|
| 550 c. c. bottle | = | .03 per cent $\text{CO}_2$ . |
| 450 c. c. bottle | = | .04 per cent $\text{CO}_2$ . |
| 350 c. c. bottle | = | .05 per cent $\text{CO}_2$ . |
| 300 c. c. bottle | = | .06 per cent $\text{CO}_2$ . |
| 260 c. c. bottle | = | .07 per cent $\text{CO}_2$ . |
| 225 c. c. bottle | = | .08 per cent $\text{CO}_2$ . |
| 200 c. c. bottle | = | .09 per cent $\text{CO}_2$ . |
| 185 c. c. bottle | = | .10 per cent $\text{CO}_2$ . |
| 100 c. c. bottle | = | .20 per cent $\text{CO}_2$ . |

**45. Composition of Expired Air:** The excess of  $\text{CO}_2$  and the deficiency of  $\text{O}_2$  may be demonstrated by two very simple experiments:

1. *Excess of  $\text{CO}_2$ :* Two flasks containing freshly filtered lime water are arranged like a Müller valve so that the air inhaled into the lungs passes through one flask, while that exhaled from the lungs passes out through the lime water of the other flask. After a few respirations the lime water in the flask through which the exhaled air passed, showed pronounced turbidity, while that in the other one remained perfectly clear, although the same volume of air was drawn through it.

2. *Deficiency of Oxygen:* Some of the air exhaled from the lungs is collected in an inverted flask that was previously filled with water. Observe that when a lighted taper is thrust into the air of this flask the flame is extinguished at once because there is not enough  $\text{O}_2$  to support combustion.

**46. Analysis of Expired Air:** The air exhaled from the lungs in ordinary respiration is collected over acidulated water. A definite quantity (100 c. c.) of this expired air is measured in a Hempel burette. It is then forced into a Hempel gas pipette filled with a very concentrated solution of  $\text{KOH}$ . It should remain there for some minutes and the pipette should be agitated so that the  $\text{CO}_2$  may be absorbed. The air is then returned to the burette; the pressure is equalized and the diminution in volume indicates the amount of  $\text{CO}_2$  present. Record the result expressed in per cent by volume.

The air is then forced into a gas pipette containing a concentrated solution of pyrogallie acid and  $\text{KOH}$  to absorb the  $\text{O}_2$  as



before. The reduction in volume shows the amount of  $O_2$  present in expired air. Record the volume per cent as before.

The *respiratory quotient* should be determined from the amount of  $O_2$  absorbed and the  $CO_2$  given off in a considerable period of time. In the analysis you have seen, compare the composition of the inspired and expired air and determine the loss of  $O_2$  and the gain of  $CO_2$ . What is the ratio between the two, i. e., what is the respiratory quotient or  $\frac{CO_2}{O_2}$ ?

**47. Analysis of Supplemental Air:** The exhaled air used for analysis in the preceding experiment represents the tidal air or that moved in and out of the lungs with each ordinary respiration.

The stationary or alveolar air contains a higher percentage of  $CO_2$  than the tidal air. A part of the air which is stationary in the lungs with ordinary respiration may be collected by making several forced expirations after ordinary expirations: the air is forced through a long piece of rubber tubing (12 feet in length) with a T tube near the mouth; from the side arm of the T tube some of the supplemental air is collected over acidulated water and analyzed by the same method as in the last experiment. Record the result.

**48. Presence of Organic Matter in Expired Air:** Two Woulff bottles, containing some colorless  $H_2SO_4$ , are arranged so that the inspired air is drawn in through one and the expired air is forced out through the other. After one breathes through these bottles for some time (15 to 20 minutes or more), observe that the acid through which the expired air has been passed is darkened slightly as shown by the brownish color compared with that in the other bottle. This charring is due to the presence of organic matter in the expired air.

The same thing may be shown by the bleaching of a weak alkaline solution of potassium permanganate.

**49. Effect of Breathing Air Vitiated by Respiration:** A number of bell jars containing ten or twelve pigeons (or more if necessary) are arranged in series so that the air is drawn from one to the other by a Chapman or Crowell suction pump for an hour or two. Only the first pigeon gets fresh air; each of the





succeeding pigeons breathe the air that has been vitiated by the other pigeons ahead of him. When the air moves slowly it is found that the air entering the last bell jar (jar A) contains about 17 to 18 per cent of  $O_2$  and 3.5 to 4 per cent of  $CO_2$ .

Observe the objective symptoms of the pigeon in the last jar (A), i. e., the effects of the respiratory impurities given off by the other pigeons in the series. Note that the animal not only suffers from hypernea and dyspnea, but that there is dullness, stupor, closing of the eyes and ruffling of the feathers.

The air from the last jar (jar A) is divided by means of two Y-tubes. Part of it is drawn through a Woulff bottle or cylindrical percolater placed on its side and containing soda lime to absorb the  $CO_2$ , and then enters jar B containing another pigeon.

Another part of the air from jar A is drawn through a Woulff bottle or percolater containing pumice stone soaked in  $H_2SO_4$ , to absorb the organic matter and moisture, and then it passes through jar C.

A third part of the air from jar A is drawn through a condenser consisting of a coil of metal tubing surrounded by a mixture of ice and salt to reduce the temperature of the air and condense some of the moisture before it enters jar D. The temperature of each jar is taken by thermometers inserted in T-tubes at the top of the jars.

Observe the behavior and objective symptoms of the pigeons in jars B, C and D, and compare them with the one in jar A. Record your observations.

1. Does removal of the  $CO_2$  (pigeon B) prevent the dullness and evident discomfort from which the pigeon A suffers?

2. Does the high percentage of  $CO_2$  alone, without the moisture and organic matter (pigeon C), produce all the symptoms seen in pigeon A, or only the dyspnea?

3. Does the reduction in temperature with its accompanying condensation of moisture in jar D, without changing the chemical composition of the air, lessen the discomfort of the vitiated air as compared with the pigeon in jar A?

4. From this experiment what would you conclude concerning the cause of the unpleasant effects of the air of an overcrowded and poorly ventilated room?



**50. Effect of an Artificial Atmosphere Containing an Excess of  $\text{CO}_2$  and a Deficiency of  $\text{O}_2$ :** A twenty-gallon gas tank is filled with an artificial atmosphere, in which the percentage of  $\text{O}_2$  is reduced by mixing with nitrogen and the  $\text{CO}_2$  is increased by adding  $\text{CO}_2$  derived from the decomposition of marble by an acid, until the  $\text{O}_2$  and  $\text{CO}_2$  are present in the same proportion as in the last experiment (i. e., about 16 per cent of  $\text{O}_2$  and 3.5 to 4 per cent of  $\text{CO}_2$ ). This is slowly drawn through a bell jar containing a pigeon. Observe that this pigeon does not show the same objective symptoms when breathing the artificial atmosphere from a chemical source as the pigeon in jar A in the last experiment, which breathed air vitiated by respiration. Describe the result.

**51. Effect of Confining an Animal in a Small Space and in a Relatively Large Space:** (1) One pigeon is placed in a small closed jar with a capacity of two liters, and kept there until distinct symptoms manifest themselves. Observe that, in addition to hypernea and dyspnea, the pigeon is restless the pupils are dilated, and there is a condition of excitement and activity. (These results appear to be due to the deficiency of  $\text{O}_2$ , as all the available  $\text{O}_2$  of the air of the jar is used up.)

(2) Another pigeon is placed in a large bell jar with a capacity of ten liters and kept there five times as long as the first pigeon. Although the time of confinement is in the same proportion as the space, observe that the objective symptoms of the second pigeon are quite different from those of the first one. In addition to dyspnea there is dullness, drowsiness, and ultimately there would be coma, but no excitement or activity. The second pigeon seems to suffer more from the gradual accumulation of  $\text{CO}_2$ , as there is still sufficient  $\text{O}_2$  present to support life.

**52. Tolerance to Foul Air (Bernard's Experiment):** A small space containing a pigeon is placed in communication with the large bell jar used in the last experiment and the air of the two jars thoroughly mixed.

Observe that the second pigeon, coming suddenly into an atmosphere greatly vitiated by respiration, seems to suffer greater dyspnea and discomfort than the first one. Is there a tolerance to foul air?



Do the occupants of an overcrowded and poorly ventilated room notice the respiratory impurities, and suffer as much discomfort as those who suddenly come into such an atmosphere from the pure air?

**53. Breathing Artificial Atmospheres:** 1. The percentage of  $O_2$  is reduced by diluting air with nitrogen in a gas tank. The artificial atmosphere is then slowly drawn through a bell jar containing a pigeon.

(a) Observe that an atmosphere containing approximately 15 vol. per cent of  $O_2$  does not produce any objective symptoms or evidence of discomfort.

(b) An atmosphere containing approximately 10 vol. per cent of  $O_2$  causes distinct hypernea and dyspnea.

2. An artificial atmosphere containing an excess of  $CO_2$  is prepared by adding  $CO_2$ , derived from the decomposition of broken marble by a mineral acid, to the air in different proportions.

(a) Observe that an atmosphere containing as much as 2 per cent of  $CO_2$ , *derived from a chemical source*, does not cause symptoms or evidence of discomfort.

(b) An atmosphere containing from 3 to 5 per cent  $CO_2$  may not cause more than hypernea and dyspnea, if the proportion of  $CO_2$  has been increased *gradually*. If an animal is suddenly placed in an atmosphere containing 5 vol. per cent of  $CO_2$  there may be struggling, and at times syncope and unconsciousness, in addition to the disturbance of respiration.

More than 5 per cent of  $CO_2$  is distinctly dangerous, as the elimination of  $CO_2$  from the blood is seriously interfered with, and may prove fatal. If the proportion of  $CO_2$  is increased very gradually an animal may live for a short time in an atmosphere containing as much as 10 per cent by vol. of  $CO_2$ .

(c) Compare these proportions with the vitiation of the air by respiration. What degree of vitiation, as indicated by the volume per cent of  $CO_2$ , is sufficient to produce distinct subjective symptoms from "crowd poison" in a crowded room? What is the permissible limit of respiratory impurities for good ventilation?

**54. Blood Gases:** Gases will be extracted from the blood by a mercury pump. After exhaustion of the blood bulbs, they





are weighed and blood is drawn into them directly from the artery and from the vein of a dog. Some blood serum is placed in another blood bulb. The amount of blood is determined by weight and the volume calculated from that.

The gases are collected in eudiometers and analyzed by absorbing the  $\text{CO}_2$  with caustic soda and the  $\text{O}_2$  with pyrogallie acid. Correction for temperature and pressure is made.

1. Record the volume of *arterial blood* taken and the volume per cent of oxygen,  $\text{CO}_2$ , and nitrogen, respectively.

2. Record the volume of *venous blood* and the volume per cent of each gas extracted from it.

3. Record the volume of blood serum and the volume per cent of gases extracted from it.

4. Observe that a solution of  $\text{NaHCO}_3$  in distilled water does not give up its  $\text{CO}_2$ , when exposed to a vacuum, as readily as blood serum. Explain the difference.

From what you have seen, write a brief summary of the difference in the proportion of the gases of arterial blood, venous blood and blood serum. How do  $\text{O}_2$  and  $\text{CO}_2$  exist in the blood?

**55. The Volume of the Air Respired as Measured by the Spirometer:** 1. *The Tidal Air:* The amount of air expelled from the lungs by each expiration in normal, quiet expiration is measured by breathing into the spirometer. The average of several observations is taken. This is usually 25 to 30 cu. inches or 400 to 500 c.c.

2. *The Supplemental or Reserve Air:* This is the amount that can be exhaled by a forced expiration, after the tidal air has been expelled by natural expiration. The average amount is 90 to 100 cu. in. or 1500 to 1600 c.c.

3. *The Complemental Air:* This is the greatest amount that can be taken into the chest by the greatest inspiratory effort, after the tidal air has been drawn into the lungs. It may be determined directly by filling the spirometer with air and measuring the amount that can be inspired after a natural inspiration. It varies in different individuals, usually being from 100 to 120 cu. in. or 1600 c.c. to 2000 c.c.

4. *The Residual Air* is the amount that remains in the lungs





after the greatest expiration. It is estimated to be 50 to 60 cu. in. or 800 to 1000 c.c.

What proportion of the air in the lungs is replaced by fresh air at each respiration, i. e., what is the co-efficient of ventilation of the lungs or the proportion between the tidal air and the *stationary air* (residual plus the reserve air)?

**56. The Vital or Respiratory Capacity:** This is the sum of the complemental, tidal and supplemental or reserve air. It is the greatest amount that can be expired after the deepest inspiration and is easily measured with the spirometer. It usually amounts to 225 to 240 cu. in. or 3700 c.c. to 4000 c.c. in men, but is less in women. It is greatest in tall people and in those accustomed to physical exercise.

Record your own vital capacity, and from the observations on different members of the class, write a brief summary of the influence of stature, body-weight, sex and active physical exercise upon the respiratory capacity.

**57. Chest Expansion:** With a tape measure take the girth of the chest at the level of the fifth or sixth rib (a) after the greatest expiratory effort and (b) after the deepest inspiration. The difference between the two gives the greatest chest expansion. It varies from 2.5 to 4.5 inches in different individuals. Women have relatively a greater chest expansion than men, as the result of thoracic breathing, although the respiratory or vital capacity is less in women than in men.

From the observations upon different members of the class, note that there is no constant relationship between the chest expansion and the respiratory or vital capacity. Describe the influence of stature and of physical exercise upon the chest expansion.

**58. Intrathoracic and Intratracheal Pressure:** A cannula is introduced through the intercostal muscles of an etherized dog into the pleural cavity and connected with a water manometer to measure the intrathoracic pressure. Another cannula is pushed into the trachea between the cartilaginous rings and connected with another water manometer to register the pressure within the trachea.

1. Observe that the *intrathoracic pressure* is always negative,



even at the end of natural expiration, and that the negativity increases with each inspiration. Record the average readings of the manometer at the end of expiration and during inspiration. Describe the cause of (a) the negative intrathoracic pressure when the chest is at rest, and (b) of the change with each inspiration.

2. Observe that the *intratracheal pressure* is negative during inspiration and positive during expiration. (This is also true of the intrapulmonary pressure.) Record the registration of the manometer and explain the cause of the changes of pressure in the different phases of respiration.

3. Observe the effects of obstructing the upper air passages (by pressing the ether cone tightly over the nose) upon the intratracheal and the intrathoracic pressure. Record the results.

4. Observe the pressure within the trachea and within the thorax after the trachea is opened. Record the result.

5. When the tracheal tube is obstructed by placing the finger over it, to what extent may the intratracheal and intrathoracic pressure be varied by the labored respiratory efforts? Record the results and write a brief account of the mechanism of natural respiration.

**59. Graphic Record of Respiratory Movements and Relation to Respiratory Sounds:** The stethometer may be used to show the changes in the different diameters of the chest with respiration, but the stethograph or pneumograph is the most convenient means of recording the respiratory movements in man. This is essentially a receiving tambour. It is fastened tightly about the chest, so that the movements of the chest wall in inspiration and expiration are easily recorded on a moving drum by means of a recording tambour. An electro-magnet is brought to write directly under the recording tambour. This is connected to a couple of dry cells with a simple key for marking the time of the respiratory sounds as one auscults the chest.

The drum is set at slow speed. One person auscults the chest and with one hand on the key marks the beginning and end of the vesicular sound by making and breaking the current. Care should be taken to get a tracing of natural, quiet respiration as the normal. Take a time tracing of seconds or fifths of seconds with the electro-magnet.



1. Observe that the expansion of the chest, produced by active muscular contraction during the phase of inspiration, is shorter than the passive return of the chest wall during the phase of expiration.

2. Observe that there is no pause between inspiration and expiration, but there may be a pause after each expiration if the respirations are slow.

3. Observe that the vesicular sound begins early in the phase of inspiration and continues into the phase of expiration. The inspiratory portion of the vesicular sound is much longer than the part that occurs in the phase of expiration. The expiratory part of the vesicular sound only occupies the first third of the phase of expiration.

Write a brief statement of your observation as to the relative time occupied by the respiratory movement and the respiratory sounds.

**60. Forced Breathing and True Apnea:** (a) Take a tracing of normal respiration. The subject then breathes 18 times per minute, taking care to draw *deep inspirations but not to make active expirations*. After 30 or 40 seconds the voluntary effort is discontinued and the respiration is allowed to occur involuntarily. In typical cases of apnea the respiration ceases altogether for 10-30 seconds, or the respirations become very shallow. This is due to the decreased  $\text{CO}_2$  in the blood (acapnea) from the deep forced respirations. As soon as it accumulates up to the normal the respiratory center is stimulated to restore the usual depth and rate of respirations.

If the subject breathes too rapidly, or if the muscular exertions are too vigorous, especially in expiration, the production of  $\text{CO}_2$  may be increased to such an extent that the  $\text{CO}_2$  of the blood is not decreased, and apnea does not occur.

(b) *Prolonged Forced Breathing* for two or three minutes causes a longer period of apnea toward the end of which slight cyanosis of the lips may occur from the want of oxygen. This may cause irregular respiration for several minutes after the period of apnea. Prolonged forced breathing may cause "light-headedness", shivering, and muscular weakness.

(c) *Forced Breathing Without Excessive Elimination of  $\text{CO}_2$* : A large paper bag is held tightly over the nose and mouth





while the subject does forced breathing, as before. The peculiar and disagreeable sensations of acapnea do not occur, as re-breathing the same air prevents excessive loss of  $\text{CO}_2$ . The voluntary effort is less than in the previous experiments. The subject does not experience apnea and may even be unable to stop breathing voluntarily. This shows that the apnea in (a) and (b) was due to an alteration in the blood gases and not to a nervous reflex (vagal apnea).

### 61. Voluntary Apnea.

1. The subject holds his breath after a moderate (normal) inspiration. The breaking point usually occurs after about 40 seconds.

2. The breath is held after a very deep inspiration. A longer time elapses (usually 50 or 60 seconds) before the breaking point is reached.

3. The subject performs forced breathing for a couple of minutes. The breath can then be held for two or three minutes.

4. The subject performs forced breathing for a couple of minutes, but this time in the paper bag. Note that the breath cannot be held as long as before.

Take tracings of all the above. Take time tracings in seconds in every experiment.

**62. Asphyxia:** When the entrance of air into the lungs is prevented in any way, or when the blood is not oxygenated, the following stages of asphyxia appear:

1. A *stage of hypernea* lasting about one minute. The respirations are increased in frequency and in depth. The inspiratory phase of the excited respiratory movements is especially prominent. Great variations of the blood-pressure occur with the efforts at respiration and the heart beats rapidly.

2. A *stage of dyspnea* and convulsions. Respiration is labored and the movements become convulsive. During the second stage the expiratory movements become more prominent. There is extreme cyanosis; the pupils are widely dilated; the struggling movements pass into irregular spasms from stimulation of the nerve centers. The second stage lasts about one minute.

Cardiac inhibition is present from stimulation of the cardio-



inhibitory center by the venosity of the blood; the arterial pressure usually rises, in spite of the cardiac inhibition, from stimulation of the vasoconstrictor center. When general convulsions occur these contribute to the rise of pressure.

3. The *third stage* is one of *collapse* which follows the irregular spasm of the second stage. Consciousness and reflex activity are lost and there is a condition of complete relaxation. Respiratory movements occur at long and irregular intervals, in which violent inspiratory efforts are made. The blood-pressure is low as the result of paralysis of the vasomotor center. The heart continues to beat for a considerable time after the respiratory movements cease. For this reason prompt efforts at resuscitation may be successful.

The third stage lasts two or three minutes or longer. In adults recovery does not occur after more than four or five minutes of complete asphyxia or suffocation, but young animals may recover after a longer period. In resuscitating a person or an animal it may be necessary to keep up artificial respiration for twenty to thirty minutes, or even longer, before spontaneous respirations occur.

**63. Artificial Respiration:** During the third stage of asphyxia, or after the respiratory movements cease from any cause, the head is lowered and the feet elevated; the mouth and pharynx are cleared; the tongue is pulled forward rhythmically (Laborde's method) to stimulate the respiratory center reflexly, traction being made during the inspiratory phase of artificial respiration.

In animals air is expelled from the lungs by pressure upon the ribs. These active respiratory movements alternate with the pulls made upon the tongue. The elasticity of the chest wall causes the thorax to expand and air to enter the lungs.

In man the Sylvester method consists in expanding the chest (i. e., producing inspiration) by drawing the arms up over the head, the tongue being pulled forward. The expiratory movement is produced by pressing the patient's elbows against the sides of the chest. The combined Laborde-Sylvester method gives the best results.

When artificial respiration must be carried on by one person it is best to place the patient face down and compress the **thorax**



by pressure applied postero-laterally, allowing the thoracic wall to expand by its own elasticity (Schäfer).

Write a brief description of the restoration of the circulation and respiration by maintaining artificial respiration, giving the time occupied.

### NERVOUS MECHANISM OF RESPIRATION.

**64. Motor or Efferent Nerves: The Phrenics:** Observe the contractions of the diaphragm from stimulation of one phrenic nerve. Note the effect of cutting one phrenic; of stimulating the peripheral end.

Note the effect of cutting both phrenics. What change is there in the type of respiratory movements after section of both phrenics?

*Recurrent or Inferior Laryngeal:* Observe the condition of the vocal cord of the same side during stimulation of this nerve. How does irritation or paralysis of this nerve change the voice? What is the condition of the vocal cords in coughing?

**65. Afferent Nerves of Respiration: Apnea:** In a normal animal, with all the nerves intact, observe the temporary cessation of the respiratory movements after rapid inflation of the lungs. Explain the cause of this apnea.

Observe the effect upon the respiratory movements of stopping the respiration by occluding the tracheal cannula at the end of inspiration. Explain the result of distending the chest.

In the same way observe the effect of suddenly stopping respiration at the end of natural expiration. Explain the result.

*Respiratory Rate and Volume Before and After Section of the Vagi:* The normal respiratory rate per minute is counted and recorded. The volume of air exhaled from the lungs for periods of two or three minutes is measured by the spirometer. An average of several observations is taken and recorded.

Later in the experiment, after both vagi have been severed, the respiratory rate and volume are again determined. Record the results. Observe that the volume is practically the same, although the rate is greatly reduced. Explain the cause of the slow respiration after the section of the vagi.



*Stimulation of the Nasal Branches of the Trigeminal Nerve:* Observe the inhibition of respiration from stimulation by blowing tobacco smoke or some irritating vapor into the nose of an animal. What is the cause of the inhibition of respiration early in anesthesia? How may this condition be distinguished from the cessation of respiration caused by the depressant effect of the anesthetic upon the respiratory center?

*Superior Laryngeal Nerve:* (a) What results from stimulation of this nerve with a weak current? (b) With a strong current? In what phase is respiration inhibited?

*Sensory Nerves:* Observe the increase in rate and depth (acceleration) of the respirations from stimulation of any mixed spinal nerve containing sensory fibers, as the central end of the cut sciatic.

*Respiratory Fibers of the Vagi:* Note the effect of cutting (a) one vagus and (b) of cutting both vagi upon the respiratory rate.

Observe the effect of stimulating the central ends of the severed vagi, beginning with a very weak current and gradually increasing its strength. Record the result. What phase of respiration is especially affected?

What is the effect of suddenly stimulating the central ends of the vagi with a strong current? Which phase is particularly affected? What kinds of respiratory fibers exist in the pneumogastrics?

Observe the result of occluding the tracheal tube at the end of inspiration and at the end of expiration, respectively, and compare with the result obtained by the same procedure before the vagi were cut. Explain the difference.

Inflate the lungs rapidly and observe whether apnea can be produced as readily after section of both vagi as can be done when they are intact. Explain the result.

From this experiment write a brief description of the respiratory functions of the afferent fibers of the vagi from the lungs, telling how they may be demonstrated experimentally and how they are stimulated under normal conditions during natural respiration.





## LYMPH AND CHYLE

A dog has been fed on fat meat several hours before the experiment. The animal is etherized; the thoracic duct is exposed just before it empties into the jugular vein and a cannula is tied in it; another cannula is placed in the principal lymphatic trunk coming from the head.

**66. Appearance of Lymph and Chyle:** Observe the milky chyle as it drops from the cannula in the thoracic duct, and compare it with the colorless lymph which comes from the lymphatic trunk of the head. Explain the difference.

Study the appearance of a drop of chyle under the microscope and compare it (a) with a drop of milk and (b) with a soap emulsion made by shaking a rancid oil with a solution of sodium carbonate. Judged by the minute division and uniformity in the size of the fat globules, how does chyle compare with the other emulsions? How does the emulsification of fats in the chyle compare with that which occurs in the intestine during the digestion of fats?

Observe the influence of inspiration and expiration, respectively, upon the flow of chyle. Explain the difference in the two phases of respiration. Count the number of drops of lymph and of chyle for periods of two minutes and record them in separate columns in a protocol of the experiment. Make two observations for the normal.

**67. Massage:** Count the number of drops from the cervical lymphatic during massage of the corresponding side of the head and neck. Record the result of two observations. From this experiment what do you consider the greatest benefit of local massage?

**68. Lymphagogues:** These are substances which cause an increase in the formation and flow of lymph when they are injected into the blood. A study of their action gives a better understanding of the formation of lymph and the conditions which influence it.

There are two classes of lymphagogues: (1) *Crystalloids*, like dextrose, urea, sodium chloride, etc., which increase the



lymph by producing a condition of hydremic plethora in the blood and a consequent increase in the blood pressure in the capillaries. If the plethora be prevented by bleeding, the hydremia alone does not cause an increase in the formation of lymph.

(2) *Colloidal lymphagogues*, like commercial peptone, egg albumin, extracts of leech heads, crayfish, mussels, etc., and curare, which seem to act by increasing the permeability of the vessel walls. With the exception of curare, they appear to act chiefly upon the blood capillaries of the liver, while the crystalloid lymphagogues act uniformly upon all the vessels of the body.

A definite amount of blood is taken from the dog. Observe that the flow of lymph and chyle is diminished as a result.

Clotting of the blood is prevented by receiving it directly into a 2.5 per cent solution of sodium citrate in the proportion of 10:1, so that a mixture of 0.25 per cent citrated blood is obtained. This is returned to the veins and the flow of lymph and chyle is determined by counting the drops for two-minute periods as before. The small amount of sodium citrate used to prevent coagulation of the blood causes very little, if any, increase in the flow of lymph.

The same amount of blood is withdrawn a second time and mixed with the citrate solution in the same proportion.

Sufficient dextrose is then injected intravenously to abstract from the tissues, by its osmotic pressure, an amount of fluid equivalent to the quantity of blood removed by bleeding. Observe that the flow of chyle is not increased, or if so only slightly, although the blood has been made hydremic by the water attracted from the tissues by the dextrose. Record the number of drops for two-minute periods as before.

After waiting five or ten minutes, the citrated blood will be injected into the vein at the temperature of the body. The flow of lymph may be increased for a short time. As soon as it returns to the normal, dextrose is again injected in exactly the same quantity as before. Observe the great increase in the flow of lymph from the lymphatic from the head, and of chyle from the thoracic duct. Count the number of drops again for two minute periods and record the results.



In this case there is a sudden plethora in addition to the hydremia. Crystalloid lymphagogues seem to act upon all the vessels of the body by physical forces to a large extent, the increase in the capillary blood pressure causing an increase in the formation of lymph. This is not the sole cause of the increased lymph flow, since it lasts longer than the increase of venous pressure. When the crystalloid lymphagogues are injected shortly before the death of an animal, the increased lymph flow continues long after the death of the animal and may reach its maximum a considerable time after the capillary pressure ceased to exist.

After the flow of lymph and chyle has subsided to the normal, a solution of commercial peptone (0.1 gram Witte's peptone per kilo of body weight) is injected intravenously. Observe that the flow of lymph from the head is not changed, but the flow of chyle from the thoracic duct is greatly increased. Count the number of drops for two-minute periods and record the result as before in a protocol of the experiment.

It has been found by experiment that the increased flow of lymph from the thoracic duct after the injection of peptone comes from the liver; also that the lymph is more concentrated and contains a higher proportion of solids in solution, whereas the lymph formed after the injection of crystalloids is more dilute and watery than the normal.

These facts indicate that colloidal lymphagogues do not act by known physical laws, but that the endothelial cells lining the blood capillaries of the liver become more permeable, probably through some change in their vital properties.

**69. Lacteals:** At the end of the experiment, when the abdomen of the dog is opened, observe the lymphatic vessels, or lacteals, of the intestinal wall and mesentery; observe their relation to the lymph glands of the mesentery.

**70. Absorption of Crystalloids and of Colloids from the Tissues:** A dog is etherized; through a lumbar incision the ureter is drawn out and a cannula tied in it so that the urine may be collected as it comes directly from the kidney. The normal urine is first collected and examined to be certain that albuminuria does not exist.





A solution of egg albumin (as a type of a colloid) is injected subcutaneously into one thigh; a solution of potassium ferrocyanide (as a type of crystalloid) is injected into the other thigh. Both of these substances are eliminated by the kidneys within two minutes when they are injected into the blood. The presence of potassium ferrocyanide may be detected by the Prussian blue test; egg albumin may be recognized by heating and acidulating the diluted urine or by the ring test with  $\text{HNO}_3$ .

Note the exact time at which the injection is made and make a protocol of the experiment. Observe that the crystalloid appears in the urine in a few minutes (usually about 8 or 10 minutes), while the colloid (egg albumin) can not be detected in the urine within an hour or more after it has been injected hypodermically.

The marked difference in the time required for absorption suggests that crystalloids are absorbed by direct diffusion from the lymph spaces through the thin capillary walls into the blood. Alkaloids are also probably absorbed in that way.

Colloids are probably taken up from the lymph spaces with the lymph and carried by the sluggish lymph stream until they reach the blood through the thoracic duct.

The difference in absorption of colloids and of crystalloids may also be shown by injecting a solution of carmine, as a type of a relatively non-diffusible pigment, and methyl blue as a diffusible substance. The color of these substances enables them to be recognized readily, both in the lymph and in the urine.

**71. Absorption of Foreign Particles from the Large Serous Cavities:** An emulsion of a finely divided insoluble pigment (water color lampblack in physiological salt sol.) is injected into the peritoneal cavities of two rabbits. One rabbit is killed two hours after the injection and the other after twenty-four hours.

(a) Observe that the lampblack collects in lines on the omentum and on the diaphragm and that it can not be washed off except by using violence. Note that the mediastinal lymphatic glands are colored black by the pigment granules, even two hours after the injection.



(b) Observe that at the end of twenty-four hours there is no free pigment in the abdominal cavity.

(c) Study microscopic specimens of the omentum from rabbits after the injection of lampblack. Note that the pigment particles are held on the surface of the omentum by a fibrinous exudate; after twenty-four hours the pigment granules are found in the large cells ("trailers") of the omentum.

(d) Examine microscopic sections through the mediastinal lymph glands of rabbits killed two and twenty-four hours, respectively, after the intraperitoneal injection of lampblack. Observe the distribution of the pigment granules in each and describe the appearance of the section of each gland.

This experiment has an important practical bearing upon the phenomena of absorption in cases of peritonitis and also upon the function of the omentum. The fact that the omentum picks up foreign particles and holds them in its meshes and cells, shows that it has a protective function in localizing infections similar to that of the lymphatic nodes.

The rapidity with which foreign particles pass out of the abdominal cavity into the thoracic lymph glands explains the widespread general disturbances which occur so soon when infectious material escapes into the peritoneal cavity, as from perforation of the intestine, vermiform appendix, etc.



## THE CIRCULATION.

### THE HEART.

**72. Arrangement of the Muscle Fibers of the Walls of the Heart:** A beef's heart has been boiled for two hours to loosen the muscle fibers by changing the connective tissue to gelatine and dissolving it. In the thin auricular walls it is difficult to separate the layers, but there is an external transverse and an internal longitudinal layer. Observe the way in which the veins open into the right auricle and the great abundance of muscle fibers surrounding the mouths of these veins.

In the walls of the ventricles three distinct layers may be made out: (a) An external spiral or oblique layer; (b) a middle transverse or circular layer; and (c) an inner longitudinal layer. Tear the fibers of the thin external layer and note the spiral arrangement, downward and to the left: observe the "figure of eight" turn by which they become continuous with the internal layer and form a whorl at the apex.

Note that the middle transverse layer of fibres is much thicker than the other two, especially at the base of the ventricles. This is a matter of extreme importance because it serves to lessen the size of the auriculo-ventricular orifices during systole, when the valves are closed, thereby rendering them competent. Degeneration of the heart muscle may result in incompetence of these valves without any disease of the valves themselves.

Observe that the inner longitudinal layer extends into the muscular columns and papillary muscles of the ventricular wall. Compare the relative thickness of the walls of the two ventricles. Record the result of this observation and explain why the wall of the left ventricle is so much thicker than that of the right side.

**73. The Auriculo-Ventricular Bundle or the Bundle of His:** This can readily be seen in the heart of the sheep, calf or ox. In the heart of other mammals this bundle can not be seen readily by the unaided eye, but it may be traced by histological examination. Examine microscopic sections of this bundle.



Observe the origin of the auriculo-ventricular bundle beneath the endocardium of the right auricle; trace it beneath the tricuspid valve into the ventricular septum and observe that it divides into two parts, one of which is distributed on the inner surface of each ventricle.

This bundle consists of special muscle fibres which conduct the contraction wave and form a continuity of structure between the auricles and ventricles. It was formerly thought that the fibrous auriculo-ventricular ring completely separated the muscular wall of the auricles from that of the ventricles in the mammalian heart.

**74. Valves and Orifices of the Heart:** Observe the arrangement of the two sets of auriculo-ventricular valves, and the semi-lunar valves; the direction in which they open, their size, attachments, etc.

Compare the size of the orifices.

Observe the origin and distribution of the coronary arteries.

**75. Change in Shape of the Heart During Action:** This is shown by hearts that have been arrested and fixed while in systole and in diastole. Note (1) that in systole the antero-posterior and transverse diameters are shortened, and that the long axis of the heart is also somewhat shortened, though to a much less extent. Observe (2) that during systole the apex becomes more pointed and that the general outline of the heart is decidedly cone-shaped; (3) that in diastole the general contour of the heart is more globular and the apex less pointed; (4) that in diastole the transverse diameter is the longest; (5) viewed from the base, the contour of the heart in diastole is elliptical, while in systole it is circular.

**76. The Valves of the Heart in Action. Gad's Heart Windows:** A glass window with a side tube has been tied in the pulmonary artery of a fresh calf's heart above the semi-lunar valves; another window has been placed in the right auricle, so as to look down on the tricuspid valve from above. A rubber bulb is tied into the apex of the right ventricle to represent systole of the heart when it is pressed upon; a small electric lamp illuminates the interior of the ventricle. The fluid enters the right auricle from a pressure bottle and is pumped





out of the ventricle into the pulmonary artery against a column of fluid, which closes the semilunar valves during diastole; a tube carries the fluid from the pulmonary artery into a funnel in the pressure bottle. A bandage is tied tightly about the base of the ventricles to lessen the size of the tricuspid orifice and prevent regurgitation into the auricle. Note (a) that when the bulb is compressed (i. e., in systole) the edges of the tricuspid valve come together, closing the orifice, thus preventing the fluid from passing back into the auricle, and compelling it to pass out through the pulmonary artery; that they open as soon as the bulb is relaxed (diastole). (b) Observe the shape and disposition of the leaflets of the valve, and the action of the chordae tendineae. (c) Note that the semilunar valves open in systole and close in diastole (i. e., when the auriculo-ventricular valves are open, the semilunar valves are closed, and vice versa).

Why is the tricuspid valve incompetent in the dead heart, unless a bandage is wrapped about the base of the ventricle?

What is the function of the valves of the heart?

**77. Verdin's Model of the Heart Valves:** This is a mechanical device for showing the action of the heart valves in determining the direction of the current through the heart. Note that only one valve is open at a time (i. e., when one is open the other is closed).

**78. The Competence of the Semilunar Valves:** The pulmonary artery has been cut away from the right ventricle of a calf's heart, and connected with an upright tube. Note how well the leaflets of the semilunar valve close off the opening when the tube, reaching to a considerable height, is filled with water so as to exert pressure on them from above.

**79. To Expose the Frog's Heart:** Pith the frog by introducing a hat pin between the skull and atlas and moving it laterally. This severs the connection between the brain and spinal cord, and destroys all conscious sensation. Pin the frog out on the frog plate, ventral surface up, with U-shaped tacks. With stout scissors make a median ventral incision through the skin from pelvis to jaw; cut through the abdominal wall, avoiding the epigastric vein which runs in the median line.



Tie this vein near the pelvis. Continue the incision upward, cutting through the bony pectoral girdle and taking care not to injure the parts beneath. Pin back the flaps. Lift up the pericardium with the forceps and cut it open.

**80. General Arrangement of the Frog's Heart:** The frenum (a delicate band of connective tissue containing a vein) runs from the pericardium to the posterior wall of the ventricle. With a curved metal "seeker," pass a ligature, moistened with physiological salt solution (0.6 per cent NaCl) about this and cut it posterior to the ligature. Anteriorly, observe the *two auricles* above; the *single ventricle* forming the apex below; the two auricles being separated from the ventricle by a distinct groove, the *auriculo-ventricular groove*. Coming off from the ventricle anteriorly, and projecting in front of the auricles, is the *bulbus arteriosus* which divides into *two aortae*. Each of these divides into three vessels. With the ligature attached to the frenum, tilt up the heart and observe the *sinus venosus* which is continuous with the right auricle; it receives the blood from the single large inferior vena cava and the two superior venae cavae. The sinus, auricles and ventricle can be seen with the apex tilted up; the auricles, ventricle and bulbus arteriosus can be seen with the heart in its normal position.

**81. The Heart in Action; The Cardiac Cycle:** Observe (1) that the contraction (systole) begins in the sinus venosus, and rapidly extends in regular order to the auricles and ventricle; (2) that the period of activity (systole) in each part of the heart is followed by a longer period of rest or diastole. If the heart is beating too rapidly to make out the *sequence of events*, apply cold physiological salt solution with a pipette until the heart beats slowly. Cold slows the heart and enables one to study the cycle of events. When the heart beats slowly the entire heart may be in a condition of diastole between the periods of activity; (3) note the difference between the diastole of the entire heart and the diastole of the individual chambers of the heart; (4) does the systole of the auricles occupy as long a time as the systole of the ventricle?

Observe the pronounced *change of color* of the ventricle as it passes from diastole into systole, while there is comparatively



little change in the color of the auricles. The ventricle seems to empty itself completely with each systole while the auricles appear to contain some residual blood.

The frog's heart does not have coronary blood vessels, like a mammalian heart, but is nourished by the blood in its chambers. The *blood spaces* of the spongy ventricular wall are filled from openings on its inner surface during diastole and emptied during systole so that the change of color is much greater than in the mammal.

What alteration in the *shape* of the ventricle occurs during systole and diastole? (The frog's heart shortens in its long axis much more than the mammalian heart.)

What change in the *position* of the apex of the heart and in the position of the auriculo-ventricular groove, or base of the heart, takes place with each systole?

*Graphic Record of the Heart Beats by the Gaskell Lever:* Lift the heart by grasping the tip of the ventricle with fine forceps and tie a silk ligature as close to the forceps as possible, so that only a little of the ventricular wall at the apex is included in the ligature. By pulling on the ends of the ligature gently, so that the thread just fails to slip between the thumb and finger, it will sink in the spongy tissue of the heart sufficiently to hold without cutting through.

The long ends of the ligature are passed through a short piece of rubber tubing of large lumen for the next experiment and are then tied to the short arm of a Gaskell lever. Adjust the lever and frog-plate so that the ligature pulls in a vertical line, causing the long arm of the lever to write lightly upon the drum. The short arm of the Gaskell lever may be counterpoised.

Connect the electro-magnet with the time-marking current arranged to mark seconds, and bring it to write under the Gaskell lever. Take a tracing with the drum at moderate or slow speed.

**82. Influence of Temperature Upon the Heart Beats:** With everything adjusted as in the last paragraph, take a short tracing (four or five inches) of the normal heart beat, and record under it the room temperature. (The temperature of the frog is the same as that of the air.) Cool some Ringer's





sol. by placing a beaker of it in a pan containing cracked ice and salt. With a pipette apply the cold Ringer's sol. keeping the rubber tubing filled so that the heart is completely immersed for two or three minutes, or longer if necessary, until the rate of the heart is slowed. Continue to apply the cold Ringer's sol. during the tracing, taking care not to interfere with the writing of the lever. Take tracings when the temperature has been reduced to  $15^{\circ}\text{C.}$ ; also at  $10^{\circ}\text{C.}$ ,  $5^{\circ}\text{C.}$  and  $1^{\circ}\text{C.}$ , respectively, with the drum at the same speed each time.

Now warm some Ringer's sol. over a flame and when the temperature reaches  $30^{\circ}$  to  $32^{\circ}\text{C.}$  apply it to the heart with a pipette as before. After applying it for several minutes, take a tracing as before, with the drum at the same speed and the electro-magnet registering seconds. Heat the physiological salt sol. to  $37^{\circ}\text{C.}$  and apply it to the heart until the rate shows acceleration. Be careful that the temperature does not go above  $38^{\circ}\text{C.}$ , as a high temperature causes the heart to go into heat rigor and lose its vital properties.

What are the effects of heat and cold, respectively, upon the rate of the heart beat? Is the height of the contraction waves changed?

*Caution:* Apply the sol. long enough to change the rate each time before starting the tracing on the drum and continue to let it run while the tracing is taken.

**83. The Excised Heart:** Remove the heart lever and rubber tubing. Count the number of heart beats per minute. Lift the heart by the ligature attached to the apex and carefully excise it, including the sinus venosus, by cutting the vessels as far away from the heart as possible. Place the heart in a watch crystal and again count the rate, keeping the heart moistened with Ringer's sol.

(a) Has removal of the heart from the body changed its rate or rhythm?

(b) Has the sequence of events in the heart been changed?

(c) Does the activity of the heart depend upon nerve impulses from the central nervous system?

(d) Is the circulation of blood through the frog's heart necessary for its action?

Now count the beats of the heart again and record the rate



at the room temperature. With a pipette apply cold Ringer's sol. (at the temperature of  $15^{\circ}$  and  $10^{\circ}$  C., respectively) for several minutes and again count the rate. Then apply warm Ringer's sol. (not over  $38^{\circ}$  C.) for several minutes and count the rate. Record the number in each case. Is the excised heart influenced by heat and cold in the same way as the normal heart when it is connected with the nervous system?

Float the heart in Ringer's solution in the watch crystal; with delicate forceps pick up the sinus venosus and with fine scissors cut it off.

(a) Does the sinus continue to beat?

(b) Does the rest of the heart beat?

(c) Is the rhythm the same in the two parts?

Now sever the auricles from the ventricle by cutting through, or just below, the auriculo-ventricular groove.

(d) Do the auricles and ventricle continue to beat?

(e) If the ventricle does not beat is it capable of beating? (Determine this by pricking it with a needle or sharp forceps.) Observe that there is a single contraction for each stimulus, and not a series of contractions.

## PHYSIOLOGICAL PECULIARITIES OF HEART MUSCLE.

Note.—Before performing the experiments of the next exercise, the following phenomena in voluntary muscle will be demonstrated to the class: 1. Submaximal contractions from weak electrical stimuli. 2. The time of the single contraction or twitch (0.1 second). 3. The very short latent period (0.01 second), which is also the refractory period in voluntary muscle. 4. Tetanus caused by a rapid succession of stimuli, which cause a fusion of separate contractions. 5. Contractions caused by the galvanic current at its make and break.

**84. Extra Contractions and Refractory Period:** Pith a frog so that it can not feel any pain. Expose the heart; tie the frenum and pin the frog on the cork plate. Tie a silk ligature to the apex of the heart and then tie the free ends over the short arm of a Gaskell lever as in the last experiment.

Arrange the inductorium for single induction shocks with a simply key in the primary circuit. To the binding posts of the secondary coil fasten the wires of special electrodes imbedded



in cork. By means of pins in the cork, arrange the electrodes so that both of the looped platinum tips are constantly in contact with the heart muscle without interfering with its contractions.

Place the electro-magnet in the primary circuit by shunting from the binding posts of the primary coil for single induction shocks, and bring it to write on exactly the same vertical line as the heart-lever. This will mark the point of stimulation.

Adjust the secondary coil to such position that induction shocks are felt by the heart both at the make and break of the primary circuit. Set the drum at moderately low speed. If the heart is beating rapidly, slow it by applying cold physiological salt solution with a pipette. Take a tracing of normal beats (four or five inches on the drum). Now, with the drum at the same speed, stimulate with single induction shocks of sufficient strength to be felt by the heart. Try to apply these stimuli at different periods of the cycle of the heart's action and gradually increase the frequency of stimulation, taking care that the electro-magnet registers the point of stimulation exactly under the heart-lever.

Do all of the stimuli cause extra contractions? If not, explain why some fail to do so. What is the refractory period in the heart? Observe that after a number of extra contractions occur in rapid succession from frequent stimuli, there is a "compensatory pause" before the heart returns to its regular rhythm. This momentary slowing of the heart offsets the unusual activity from artificial stimulation. Before the paper is taken from the drum the electro-magnet should be connected with the time-marking current for recording seconds under the tracings with the drum at the same speed as above.

**85. Inhibition of the Heart by Stannius' Ligature:** With a curved seeker clear the two aortae from the auricles and pass a silk thread, moistened with physiological salt sol., between the aortae and superior venae cavae. With the ligature attached to the apex turn it up toward the head, and observe the "crescent," i. e., a V-shaped or crescentric white line, caused by a collection of nerve cells, which occurs just at the junction of the sinus venosus with the right auricle. Tie the silk ligature just over this crescent. This is the "first ligature of





Stannius." Observe that the sinus continues to beat in its normal rhythm while the auricles and ventricle stop in the phase of diastole and remain quiescent for a variable time. (The effect of this ligature will be explained more fully later, under the heading of the nervous control of the heart.)

Note.—If the ventricle starts to beat spontaneously during the following experiments, tie another ligature to the auricular side of the first ligature of Stannius described above.

**86. Staircase Contractions:** Return the heart to its normal position and fasten the ligature attached to the apex to the short arm of the Gaskell lever as before. Arrange the electrodes so that both platinum wires are in contact with the heart muscles as before. Bring the electromagnet, shunted from the primary circuit, to write exactly in the same vertical line with the Gaskell lever.

Starting with the secondary coil at right angles to the primary, stimulate with single induction shocks, increasing the strength of the current until a contraction is obtained at the break of the current. Set the drum at slow speed. Now repeat this minimal stimulus at intervals of one second or more, while the drum is moving at slow speed. Are all the contractions the same in height, or do they increase in height for several beats, forming the "stair-case contractions"? This can only be obtained after the ventricle has been inactive for a few minutes. Observe that the improvement in the degree of contraction in the "staircase contractions" occurs without any increase in the strength of the stimuli.

**87. Effect of a Weak and of a Strong Stimulus:** When no further increase in the height of the contractions is obtained by repeating the minimal break shock, increase the strength of the stimuli by gradually shoving the secondary nearer to the primary coil. Observe that all the contractions are maximal and that a gradual increase in the intensity of the electrical stimulation does not cause any greater degree of contraction than a minimal stimulus. Unlike voluntary muscle, heart muscle does not show submaximal contractions from weak electrical stimuli.

**88. Latent Period and Time of Contraction:** See that the





electro-magnet writes exactly on the same vertical line as the heart-lever when the drum moves at fastest speed. Set the drum for fastest speed and let it make one complete turn to get its maximum velocity. Stimulate with single induction shocks. Observe the long interval between the point of stimulation and the beginning of contraction, i. e., the "latent period." To measure this, connect the electro-magnet with the spring chronograph registering tenths of a second, and with the drum at the same speed as before, take a time tracing just below the other tracing. How long is the latent period in heart muscle? How much time is occupied by the periods of contraction and relaxation, respectively? Compare the time for a single contraction of heart muscle with that of voluntary muscle, giving the time occupied by the latent period, period of contraction and period of relaxation of each.

**89. Refractory Period:** Arrange the electro-magnet to mark the point of stimulation exactly under the heart-lever as before. Find a strength of current which causes contraction, both at make and at break. Set the drum for fastest speed and let it make one turn to get a uniform velocity. With the key in the primary circuit make and break the current (1) so that break shocks fall in the latent period; (2) take another tracing with break shocks following the make at longer intervals, but within the period of contraction; (3) take a third tracing with still longer intervals, i. e., let the break shock fall immediately after the crest of the contraction wave caused by the make shock, or just at the beginning of diastole. Observe that only those stimuli which fall during the relaxation are felt; those falling in the latent period and period of contraction have no effect, i. e., they fall in the "refractory period" of the heart when it is not sensitive to stimulation. How long is the refractory period?

Voluntary muscle is only refractory during the latent period; stimuli falling during the period of contraction cause a summation or summing-up of the separate contractions. Can a summation of the contractions be obtained with the heart muscle?

**90. Tetanizing Current:** Find the minimal stimulus which



causes contraction, both at make and break. Arrange the inductorium for the tetanizing current. Shunt the electro-magnet from the binding posts of the primary current. Set the drum at moderate speed and stimulate for about five seconds at a time. Does the tetanizing current produce a continuous contraction, like the complete tetanus in voluntary muscle, or a series of separate contractions? Explain the difference.

**91. Effect of the Galvanic Current:** Remove the heart-lever, turn the heart up, and with scissors, sever the ventricle from the rest of the heart by cutting through the auriculo-ventricular groove. Place the excised ventricle on the cork plate and observe that it does not beat spontaneously.

Disconnect the inductorium, but leave the simple key in the circuit from the two dry cells. Wrap the free end of each wire about a pin. Stick the pin connected with the positive pole of the battery in the cork plate so that it touches the base of the ventricle; stick the other pin in the cork so that it touches the apex, taking care not to prick the heart with the pins.

Now close the key for a few seconds at a time and observe that when the galvanic current travels from base to apex, the ventricle usually gives a series of contractions during the passage of the current.

Reverse the direction of the current by changing the wires at the binding posts of the batteries. Again stimulate for a few seconds at a time. Usually when the current passes from apex to base, contractions only occur at the make and break and not during the passage of the current. If satisfactory results are not obtained repeat the experiment with a weaker current.

(Voluntary muscle only contracts at the make and break of weak or moderate galvanic currents.)

## NERVOUS CONTROL OF THE FROG'S HEART.

**92. Inhibition by Stimulation of the Crescent:** Pith a frog and pin it out on the plate. Tie a ligature to the apex of the heart and connect with the short arm of a Gaskell lever to record the beats as in the previous experiments.



Arrange the inductorium for a tetanizing current with the electro-magnet shunted off from the primary binding posts and the hand electrodes attached to the secondary coil. Arrange the electrodes in the holder so that the platinum tips are in contact with the crescent. Starting with a weak current gradually increase its strength by shoving up the secondary coil until a strength of current is found which causes inhibition of the ventricle when the hand electrodes are applied to the crescent at the junction of the sinus venosus and right auricle. Use the minimum current which will produce this effect and be careful not to use too strong a current.

The crescent contains motor as well as inhibitory nerve cells and often inhibition can not be obtained by stimulation at the first application of the electrodes. In that case the position of the electrodes should be changed until a location is found which gives inhibition when stimulated with a current of suitable strength. When that location has been found, care should be taken not to change the position of the electrodes.

With the electro-magnet writing directly under the heart-lever, set the drum at moderately slow speed and take a tracing of the normal heart beat. Then stimulate the crescent for four or five seconds using the same strength of current as before and keeping the electrodes in the same position, so as to obtain a graphic record of cardiac inhibition. If inhibition does not occur increase the strength of current slightly, but be extremely careful that the hand electrodes are applied to the *crescent* and not to the wall of the sinus or the auricle.

Let the heart rest a few minutes. Now stimulate again.

(a) Does inhibition appear as soon as the stimulus is applied or is there a "latent period"?

(b) Does inhibition disappear the instant the stimulation ceases or is there an "after-effect"? (The after-effect may appear as partial inhibition instead of complete inhibition.)

(c) Is the ventricle inhibited in systole or diastole?

(d) Now apply 0.2 per cent atropine sulph. sol. to the heart by allowing it to fall drop by drop on the ventricle from a small pipette. After waiting a minute or two, take another tracing. What effect has the atropine produced on the rate of the heart beat?





Now stimulate the crescent with the same current used before and with the electrodes in the same position. Do you get inhibition? Explain the result.

Now connect the electro-magnet with the binding posts of the time-marking current. Set the drum at the same speed as before and take a tracing of seconds under the tracings made above.

**93. The Ligatures of Stannius Recorded by the Graphic Method:** With the heart arranged for recording the beats by the Gaskell lever, as in the last experiment, pass a silk thread beneath the two aortae and bring it in position over the crescent. Take a normal tracing with the drum at moderately slow speed. Now tie the *first ligature of Stannius*, i. e., over the crescent, just at the junction of the sinus venosus and auricle. Observe that the sinus continues to beat as before, while the auricles and ventricle stop.

Bring the lever to write very lightly on the drum and see that it is carefully counterpoised. Take a tracing of the sinus beats which shows slight waves in the same rhythm as the normal heart beats.

Now tie the *second ligature of Stannius* about the auricles, just a line or two to the auricular side of the auriculo-ventricular groove. After waiting a short time, the ventricle starts to beat, either at long and irregular intervals, or very slowly in a rhythm of its own, and quite different from that of the sinus. Take a tracing to show the sinus and ventricular beats after the second ligature. The effect of the second ligature is variable and not constant like that of the first ligature of Stannius. After waiting a reasonable time, another ligature may be applied close to the last one if the ventricle does not start to beat. Care should be taken that the second ligature is not tied in the auriculo-ventricular groove or the ventricle will not beat again spontaneously.

With another frog arranged for recording the beats with the Gaskell lever as before, take a normal tracing and then apply the Gaskell clamp about the middle of the auricles. Observe that the sinus and auricles continue to contract in the normal rhythm, while the ventricle contracts in an independent



rhythm. Several beats of the auricles occur for each beat of the ventricle. Take a tracing of these beats. Connect the electro-magnet to the time-marking current and take a tracing of seconds beneath each of the above tracings. Use this same frog for the next experiment.

**94. Influence of Temperature on the Sinus-auricular and on the Ventricular Beats:** Disconnect the ligature from the short arm of the Gaskell lever and pass it through the short piece of rubber tubing of large lumen for immersing the heart in fluid. Attach the ligature from the apex to the short arm of the Gaskell lever again and arrange it to write lightly on the drum so that the sinus auricular contractions are registered distinctly.

Cool some Ringer's sol. in a beaker by placing it in a mixture of ice and salt. When the temperature has been reduced to  $10^{\circ}$  C. fill the rubber cup from a pipette and keep the heart immersed in the Ringer's sol. for two or three minutes, or longer if necessary, until the rate of the auricular contractions is distinctly reduced. Now take a tracing with the drum at the same speed as before, keeping the heart immersed in cold Ringer's sol. from the pipette while the tracing is taken.

Take another tracing in the same way after applying Ringer's sol. at  $5^{\circ}$  C. with the drum at the same speed.

Warm some Ringer's sol. and apply it to the heart at  $30^{\circ}$  to  $32^{\circ}$  C. for several minutes as before, until there is a distinct increase above the normal rate of contraction. Take a tracing with the drum at the same speed as before, allowing the warm Ringer's sol. to flow from the pipette into the rubber cup while the tracing is taken.

Observe the influence of heat and cold, respectively, on the sinus-auricular and ventricular beats, after independent rhythms have been produced by the Gaskell clamp. If all parts of the heart come to beat at the same rate in one rhythm after warm Ringer's sol. has been applied, the Gaskell clamp may be applied again.

**95. Cardiac Inhibition from Stimulation of the Vagus in the Tortoise:** Fasten a pithed tortoise on a holder. Cut close to the



plastron in removing it. Dissect out the vagus in the neck, and place small electrodes under the right vagus.

Open the pericardium and tie a ligature to the apex of the ventricle. Attach this to a Gaskell lever with a light spring or a piece of rubber arranged above the lever to make slight tension upon the heart. Keep the lever horizontal between contractions.

Arrange the induction coil for a tetanizing current with the electro-magnet as a shunt in the primary current. Find the minimal strength of current which will cause complete inhibition of the heart. Set the drum at moderately slow speed. After taking a tracing of the normal beats, stimulate the vagus with currents of suitable strength to get (a) partial and (b) complete inhibition, noting the position of the secondary coil in each instance.

Observe carefully the effect upon the heart beats:

- (a) In what phase of the heart cycle does inhibition occur?
- (b) Does inhibition occur as soon as the stimulus is applied or is there a "latent period"?

(c) Does the inhibition (either partial or complete) continue as an "after-effect" for some time after the stimulation ceased?

(d) What is the character of the first contractions that occur after complete inhibition?

(e) After the heart returns to its normal rate, are the individual contractions any higher than the normal ones, i. e., have the contractions been improved by vagus stimulation?

Connect the electro-magnet with the binding posts of the time-marking current, and, with the drum at the same speed as before, record seconds beneath each of the above tracings.

**96. Effect of Nicotine on the Heart:** With everything arranged as in the last experiment, set the drum at the same speed and make the following observations:

1. Apply one or two drops of nicotine chloride directly to the surface of the turtle's ventricle with the drum at the same speed as before. If an excess of nicotine is avoided, there is partial inhibition from the stimulation of the inhibitory fibers within the heart. Stimulate the vagus again, using the minimal current which will cause complete inhibition.





2. Apply several drops of nicotine until the rate is increased. Now stimulate the vagus again, using the strength of current required to produce complete inhibition before. This fails to produce inhibition, as nicotine paralyzes the extrinsic nerve fibers to the heart.

Before removing the paper from the drum, take a time-tracing, marking seconds beneath each of the above tracings with the drum at the same speed as before.

This experiment, in connection with the next one, shows that nicotine probably paralyzes the inhibitory fibers or their terminations, but does not paralyze the inhibitory cells. The cells probably act as relay stations and are connected with the extrinsic inhibitory fibers by arborizations with the nerve fibers of the vagus.

**97. Action of Pilocarpine and Atropine:** 1. Stop the drum for a few minutes and apply pilocarpine muriate until the decrease in rate shows partial inhibition. Take a tracing of this with the drum at the same speed as before.

Stimulate the heart by mechanical irritation (by touching the ventricle with forceps or pithing pin). Observe that the heart is irritable during the inhibition produced by pilocarpine.

2. Apply atropine until the inhibition is overcome. Let the drum continue at the same speed as before. Stimulate the vagus again, starting with the same strength of current used before and gradually increase it. Observe that inhibition cannot be produced.

Pilocarpine stimulates the inhibitory ganglia within the heart. Atropine paralyzes these, as well as the inhibitory fibers, so that stimulation of the crescent in the frog after the application of atropine fails to produce inhibition. (See Exp., 92, d, page 142.) This can not be done in the turtle, as the inhibitory nerve cells are not grouped together at one point. These ganglion cells are paralyzed in the turtle as in the frog, and cannot be stimulated by pilocarpine after atropine has acted on them.

Before removing the paper from the drum take time tracings, marking seconds under each tracing with the drum at the same speed.





**98. Reflex Removal of Cardio-inhibitory Tone by Deglutition:** Count your partner's pulse for four (4) periods of fifteen seconds each and record the observations as the normal.

While he slowly sips cold water count his pulse again for four periods of fifteen seconds each. Record your result and compare with the normal rate.

Observe that the removal of cardiac inhibition produced reflexly by swallowing causes a considerable, but momentary, increase in the rate of the heart's action.

**99. Reflex Cardiac Inhibition from Stimulation of the Abdominal Sympathetic:** The brain of a frog should be destroyed by pithing but the medulla should not be injured. Lay the frog on its back and count the number of heart beats in one-half minute by watching the heart through the thin chest wall.

Make a small opening with scissiors through the abdominal wall to one side of the epigastric vein. With forceps draw out several loops of the intestine and with the handle of a seeker strike it repeated sharp taps while your partner counts the rate of the heart beats as before. Record the rate before and during the violent mechanical stimulation.

It has been found that the sympathetic fibers from the abdominal viscera convey these afferent impulses to the cardio-inhibitory center in the medulla.

## NERVOUS CONTROL OF THE MAMMALIAN HEART.

**100. Cardiac Functions of the Pneumogastric Nerves:** For this experiment a dog will be used. The animal is anesthetized, and the carotid arteries and pneumogastric nerves exposed by a median incision in the neck. A cannula is placed in each carotid artery. One is connected with a mercury manometer, and the other with a membrane manometer. The pens of the two manometers are arranged to write on the same perpendicular line on the large kymograph. The electro-magnet, marking seconds, is adjusted to write on the base line or abscissa of the tracing by the mercury manometer. The mercury manometer records the general changes in blood pressure and heart rate, but the inertia of the mercury prevents it from showing slight and rapid changes



in the force of individual heart beats. The membrane manometer is a more accurate means of recording changes in the force and character of the individual heart beats. The cardiac functions of the nerves are shown by stimulating them with a tetanizing current and by cutting them.

1. Observe the cardiac inhibition and fall in blood pressure that results from stimulation of these nerves. Describe the effect of stimulating both nerves (a) with a weak and (b) with a strong current. Note the increase in the size of each wave on the tracing during the inhibition. This is due to the fact that more blood is forced out with each beat (the ventricle being overfilled during the prolonged diastole) but the *force* is diminished as is shown by the fall in pressure. Note that the inhibition only lasts a short time after the stimulation is withdrawn, and that the beats rapidly return to the normal size and rate. (The "after effect" from stimulating inhibitory fibers is much less pronounced than that which follows stimulation of the sympathetic fibers.)

2. Observe that when each nerve is stimulated separately with the same strength of current, one (usually the left) produces a greater degree of inhibition than the other.

3. Observe that cutting one vagus, as a rule, produces but little change in the rate, the inhibitory control through the other vagus being sufficient to prevent marked increase in either rate or pressure.

4. Note the *reflex cardiac inhibition* when the *central end* of one cut nerve is stimulated; and the *direct inhibition* from stimulation of the *peripheral end*. This shows that the vagi contain cardio-inhibitory fibers that run in both directions—from the heart to the center (afferent) and from the center to the heart (efferent). Observe that in reflex cardiac inhibition from stimulating the central end of the cut vagus, the fall in pressure does not occur, or is very slight, and sometimes there is a rise in pressure. This is because the vasomotor center is stimulated reflexly at the same time, and by constricting the blood-vessels, prevents the fall in pressure which would otherwise occur.

5. Observe the marked rise in pressure and increase in rate that occurs after cutting both vagi. The cardio-inhibitory center exerts a constant restraining influence over the heart, the *inhib-*



itory tone, and when this restraint is removed the heart "runs away." The rise in pressure results from the increased frequency of the heart's action. Note that the rise in pressure does not last long, but disappears in a few minutes, because the blood-vessels become adjusted by dilating so as to offset the increased action of the heart.

6. Observe that when the central end of the vagus is stimulated after both vagi have been cut, there is no slowing of the heart; this shows that the vagi contain all the efferent cardio-inhibitory fibers. The rise in pressure in this case is due to stimulation of the vasomotor center.

7. Observe the action of pilocarpine in causing cardiac inhibition when injected intravenously after both vagi have been cut. This is due to direct action on the inhibitory nerve cells within the heart.

Observe the antagonistic action of atropine in removing the cardiac inhibition by causing paralysis of the inhibitory ganglion cells within the heart. Note that stimulation of the peripheal ends of the cut vagi fails to produce inhibition after the administration of atropine.

Make a protocol of this experiment, and write out a brief summary of the cardiac functions of the pneumogastrics from what you have seen.

**101. Functions of the Cardiac Sympathetic Fibers:** In dogs the sympathetic fibers that go to the heart leave the spinal cord by the second, third and fourth thoracic nerves, and pass to the *stellate* or first *thoracic ganglion* that lies on the head of the second rib; from here the cardiac fibers reach the inferior cervical ganglion in two bundles, that pass around the subclavian artery, forming the *loop or annulus of Vieussens*.

In order to show the functions of these fibers, a dog is anesthetized; the carotid arteries are connected with the mercury and membrane manometers as in the last experiment; a cannula is tied in the trachea for artificial respiration, and the first rib on each side is resected so as to expose the subclavian vessels and stellate ganglion. The loop of Vieussens can then be placed on the electrodes.

The sympathetic nerves contain both accelerator and augmen-





tor fibers for the heart. Their distribution on the two sides is variable; usually the augmentor fibers predominate on the left side and the accelerator on the right side.

1. Observe the effect of stimulating the *left* annulus of Vieussens. Note the slight increase in rate and the great augmentation in force. The augmentation is shown by the increase in the height of the waves on the tracing of the membrane manometer, i. e., the increase in pulse pressure. The mean general blood pressure, as shown by the mercury manometer, may not be increased.

2. Observe the effect of stimulating the *right* annulus of Vieussens. Note the marked increase in the rate of the heart, accompanied by some rise in pressure, and by little or no augmentation.

3. Observe the long latent period when the cardiac sympathetic fibers are stimulated.

4. Observe that both augmentation and acceleration last for a considerable time after the stimulation is withdrawn, i. e., the "after-effect" is much more pronounced from stimulation of sympathetic than that from stimulation of inhibitory fibers.

Record the protocol of this experiment and write a brief summary of the functions of the cardiac sympathetic fibers from what you have seen.

**The Mammalian Heart in Action:** At the end of the vagus experiment the chest of the dog will be opened and the heart exposed, the animal being kept alive by artificial respiration.

**102. Nature of the Contraction in Heart Muscle:** The contraction of the heart is a single twitch, and not a tetanus. This can be shown by the "physiological rheoscope," a nerve-muscle preparation from a frog. If such a preparation is held so that the nerve rests on the heart muscle, the frog's muscle will contract with a single twitch for each beat of the heart, the nerve being stimulated by the "current of action" that is developed in the heart muscle with each beat. If the heart beat were a "tetanus" (a prolonged, continuous contraction caused by a number of stimuli) the frog's leg would show a prolonged contraction, instead of a twitch that occupies only 0.1 second.



**103. Observe the Mammalian Heart in Action:** (1) Note the sequence of events in the heart while it is slowed by pilocarpine. (2) Observe the change in *shape* during contraction, especially the narrowing in the transverse diameter. Note that the whole heart becomes more pointed or cone-shaped in systole, and globular in diastole. The exposed heart is flattened out in diastole, but in the normal condition with the chest closed, this does not occur. (3) Observe the tilting up of the apex, the twisting of the heart from left to right and the descent of the auriculo-ventricular groove during contraction. (4) Observe the hardening of the heart muscle. What relation has this phenomenon to the cardiac impulse?

**104. Time of Filling of the Coronary Arteries:** Note that when a branch of a coronary artery is cut, the spurt occurs with the systole of the ventricle, just as in any other artery.

**105. Fibrillary Contraction:** This is a condition in which the individual bundles of the heart muscle contract independently of each other, so that *coördination of contraction* is lost and the heart looks like a quivering mass of jelly. Fibrillary contraction can be produced by (1) tying one of the large coronary arteries or (2) by injecting milk (or melted paraffin) into one of the large arteries to produce embolism of the terminal coronary arteries or (3) by puncturing the so-called Kronecker's "coördination center"—a spot in the ventricular septum about the junction of the middle with the basilar third.

Write out a brief account of the experiment to show fibrillary contraction.

**106. Influence of the Inorganic Salts of the Blood Upon the Heart:** Pith a frog and expose the heart. Carefully cut the pericardium away from the two aortæ, taking care not to cut the two superior venæ caviæ. Cut the frenum. Tie a silk ligature to the apex of the heart for attaching it to the Gaskell lever. Now turn the apex of the heart upward, making slight traction upon the silk ligature attached to it. Pick up the posterior layer of the pericardium with forceps and carefully dissect it away from the inferior vena cava so as to expose this vein below the point where it opens into the sinus venosus. By means of a



threaded blunt seeker pass a soft ligature about the inferior vena cava near the sinus venosus, pushing the seeker through liver tissue if necessary to get behind the vena cava. With the points of scissors make a nick in the inferior vena cava as far away from the sinus venosus as possible, and allow the blood to escape. Fill the T cannula and rubber tubing attached to it with Ringer's Sol. Close the end of the longer tube with a piece of glass rod, and apply a clip to the side tube; see that all air bubbles are excluded.

Now remove the blood and clots from about the heart by sponging with a piece of cotton moistened with Ringer's Sol. Insert the cannula into the opening in the inferior vena cava, and tie the soft ligature about its neck. After having tied the ligature about the neck of the cannula, tie the ends of the ligature around the side arm of the T cannula to keep it from pulling out. Turn the heart back to its normal position and with scissors make a nick in each aorta, close to the bulbous arteriosus.

Fasten the frog plate to the stand and adjust the ligature attached to the apex to the short arm of the Gaskell lever; arrange this to write lightly upon the drum; connect the electromagnet to the time current to mark every fifth second. Connect the rubber tubing on the long arm of the cannula with the thistle funnel, taking care that there are no twists. The funnel should be on the level with the heart or a little above it, so that when it is filled the pressure will not overdistend the heart. The fluid in the funnel should be high enough to fill the ventricle and escape from the aorta without overdistending the auricles.

1. Perfuse the heart with *normal Ringers Sol.* (NaCl 0.7 per cent,  $\text{CaCl}_2$  0.026 per cent, KCl 0.03 per cent.) Arrange the gear of the kymograph for slow speed and set the drum at such speed that the separate tracings of contractions can be counted. Take a short tracing of the normal (two or three inches).

2. Replace the Ringer's Sol. with *physiological salt solution*, Sol. C (0.7 per cent NaCl). Do not stop the drum or change anything, but mark the point at which the change of fluid was made. Observe the steady decrease in the height of the contractions when the heart is perfused with an isotonic solution.

3. As soon as the contractions cease, change the fluid to *physiological salt solution*, plus the *normal amount of calcium found*





in the blood, i. e., NaCl 0.7 per cent plus  $\text{CaCl}_2$  0.026 per cent (Sol. D). Mark the point at which the change was made but do not stop the drum or change the apparatus.

4. As soon as the height of the contractions returns to normal, observe whether or not the beats are rhythmical. Now change to physiological salt solution containing an *increased percentage of calcium*, i. e., 0.06 per cent  $\text{CaCl}_2$  (Sol. E). Mark the drum to show the point at which the change of fluids was made as before. Observe the stimulating effect of the Ca upon the height of the contractions, especially the ventricular contractions. If the tracing is continued long enough, the heart's action will become arrhythmical from the excessive amount of Ca and on account of the absence of the K. Contractions occur after incomplete relaxation of the heart muscle and "calcium spasm" may occur, i. e., more or less continuous contraction.

5. Restore the normal action of the heart again by perfusing with normal Ringer's Sol. (Sol. A).

If the heart beats are restored to normal by perfusion with Ringer's Sol. (Sol. A.) the same frog may be used for the following experiments, or a fresh frog may be used arranged for perfusion as before. In the either case take a tracing of the normal beats while perfusing with normal Ringer's Sol. (Sol. A.).

6. Without stopping the drum or changing the position of any of the apparatus, remove the normal Ringer's Sol. by opening the clip on the side arm of the T cannula, and replace it with a *modified Ringer's Sol. containing a high percentage of potassium*, Sol B (NaCl 0.7 per cent,  $\text{CaCl}_2$  0.026, and KCl 0.07 per cent). Open the clamp on the side arm of the cannula and let some of the fluid escape; if necessary fill the funnel again with Sol. B. Mark the point on the drum at which the fluid was changed.

Observe the *potassium inhibition* shown by the decrease in the rate and in the height of the contractions. As soon as this result is obtained, replace the fluid in the funnel with normal Ringer's Sol. again; let a little escape by the side tube as before, and mark the position on the drum at which the change was made. Allow the perfusion with normal Ringer's Sol. (Sol. A.) to continue long enough to remove the excess of potassium, i. e., until the heart beats become normal in rate and force.





7. Now replace the Ringer's Sol. in the funnel with a modified Ringer's Sol. (Sol. F) containing the normal amount of calcium and potassium, but no sodium. This solution is made isotonic with 0.7 per cent NaCl by using a 2.15 per cent solution of dextrose or a 4.09 per cent solution of sucrose instead of sodium chloride.

Observe that the beats of the heart gradually become weaker after the perfusion has been continued for a few minutes. Note the effect upon the muscle tone as indicated by the position of the heart lever at the end of the relaxation with each diastole.

From these observations state your conclusions concerning the important part played by NaCl in the blood and in Ringer's sol. Can it be replaced by a non-electrolyte in sufficient amount to give an isotonic solution, or does it serve some other purpose than that of maintaining a certain osmotic pressure?

**107. Influence of Drugs upon the Heart as Determined by Perfusion:** When the heart beats have been restored to normal by perfusing with normal Ringer's sol. after the preceding and each of the following experiments, make a tracing while perfusing with each of the following solutions:

(a) A one-tenth saturated solution of ether in Ringer's sol., made by adding 9 parts of normal Ringer's sol. to one part of the same solution that has been fully saturated with ether. Care must be used to keep the bottle stoppered and to prevent the ether from vaporizing.

(b) Perfuse with a one-tenth saturated solution of chloroform in Ringer's sol. made in the same way as in the preceding experiment.

Observe that ether does not weaken the heart beats in this concentration, while chloroform causes marked direct depression.

(c) Perfuse with 1 to 50,000 sol. of adrenalin in Ringer's sol. and observe the stimulating effect upon the heart muscle. The action of digitalis and stophanthus may be shown in the same way.

**108. Isolation of the Mammalian Heart:** For this experiment a dog or cat is used. The animal is anesthetized and the chest opened under artificial respiration; the heart is exposed



by opening the pericardium. The inferior and superior venae cavae are tied off, and a cannula placed in the aorta just above the semilunar valves. The heart is then cut out, and the cannula in the aorta attached to a tube leading from a bottle in which the perfusing fluid is kept under pressure. The perfusing fluid consists of Ringer's solution which has been thoroughly charged with oxygen. This is kept at bodily temperature by immersing the pressure bottle in a water bath. Sufficient pressure is maintained in the bottle to force the fluid through the coronary circulation. This is done by allowing oxygen to escape from a cylinder into the large bottle containing Ringer's solution under a pressure equal to that of the blood in the coronary arteries, viz., 75-80 mm. Hg. This pressure closes the semilunar valves, and at the same time forces the fluid through the coronary vessels of the heart. The fluid after passing through the coronary vessels is returned to the right auricle, and is collected from a cannula tied in the pulmonary artery. The heart is immersed in Ringer's solution, kept at bodily temperature. Observe the effect of changes in temperature of the Ringer's sol. Write out a brief summary of the conditions necessary for the regular action of the isolated mammalian heart. How do the conditions differ from those necessary for the activity of the frog's heart?



**THE BLOOD-VESSELS.****PHYSICS OF THE CIRCULATION.**

**109. Schema of the Circulation:** This is an arrangement of glass tubes of different sizes to show (1) that division of the tubes increases the resistance and (2) that the velocity decreases inversely in proportion as the combined sectional area of the tubes increases.

Fluid flows from a pressure bottle through a large tube 10 mm. in diameter; by means of Y's this divides into eight tubes of medium size, each 5 mm. in diameter; these in turn divide into sixteen small tubes, each of which has a diameter of 3 mm., and these divide into 36 tubes, each having a diameter of 2 mm. The tubes then unite by means of Y's at the other end so that the outflow from the system occurs through a single tube of the same size as the inflow.

Vertical tubes are placed at different points to show the pressure (a) in the larger tubes representing the arteries; (b) in the smallest tubes which represent the capillaries; and (c) in the distal tubes which correspond to the veins.

The area of transverse section of the single

large tube (10 mm. diam.).....= 78.5 sq. mm.

Combined sectional area of 8 tubes of me-

dium size (5 mm. diam.).....=157.0 sq. mm.

Combined sectional area of 16 small tubes

(3 mm. diam.) .....=113.09 sq. mm.

Combined sectional area of 36 small tubes

(2 mm. diam.) .....=113.09 sq. mm.

The frictional area, or area of contact between the fluid and wall of the tube, for a length of 1 cm. is as follows:

Single large tube (10 mm. diam.).....= 314.0 sq. mm.

Eight tubes of medium size (5 mm. diam.)...=1256.6 sq. mm.

Sixteen tubes of smaller size (3 mm. diam.)...=1523.9 sq. mm.

In 36 tubes of smaller size (2 mm. diam.)...=2261.95 sq. mm.

Observe (1) that air bubbles or suspended particles in the fluid move slowly in the small tubes, where the combined sec-





tional area is large as compared with the large tube where the sectional area is much less; (2) the pressure steadily diminishes as the distance from the pressure bottle increases; (3) the greatest fall of pressure occurs with the large number of small tubes, as division of the tubes causes a great increase in the area of contact between the fluid and the wall or the "frictional area." Make a protocol of this experiment.

**110. Flow Through Capillary Tubes:** Record the time required for a given amount of water to flow through glass tubes of the same length, but varying in diameter. Observe that the velocity of outflow is in inverse proportion to the square of the diameter if the diameter is more than 1 mm.

Contrast that with the flow in capillary tubes of 1 mm. or less in which the time of outflow is inversely in proportion to the fourth power of the diameter. Record the time for each and explain the result.

**111. The Viscosity of Blood:** Record the time required for (a) distilled water and (b) for defibrinated blood to flow through a viscosimeter. Exactly the same quantity of fluid is used at the same temperature in each case. How does the viscosity of blood influence its flow through a tube of capillary bore as compared with a limpid fluid like water?

**112. Harvard Circulation Schema:** This is a mechanical device in which the fluid is propelled by a pump with valves which works intermittently to represent the heart; a piece of bamboo, with very small channels, offers an enormous peripheral resistance; manometers are connected with the tubes which correspond with the arteries and veins respectively; elastic tubes cause a continuous flow from the intermittent pressure. A side tube with a screw clamp enables one to vary the peripheral resistance, while a changeable eccentric on the pump allows variations to be made in the force with which the fluid is expelled. Changes in the arterial pressure from increasing or decreasing the force or frequency of the beats are easily produced; also the changes in the arterial and venous pressure from increasing or decreasing the peripheral resistance; pronounced pulsations may be felt by applying the



fingers to the rubber tube near the pump, but the flow from the veins is free from pulsation if considerable resistance is interposed.

**113. Flow of Fluids Through Rigid and Elastic Tubes:** A rigid glass tube and an elastic rubber tube are connected with the two arms of a Y tube. The tubes have the same lumen and at their ends are attached tips of the same size. Observe (1) that the outflow from each tube is the same when the fluid is forced through them under constant pressure, as from a pressure bottle; (2) that when fluid is forced through the rigid glass tube intermittently, as from the pressure of a rubber bulb, the outflow is also intermittent, corresponding to the inflow; when fluid is forced into the elastic tube intermittently, the outflow is constant with spurts from each increase of pressure; (3) that the outflow from the rigid tube with intermittent pressure becomes continuous when an inverted bottle filled with air is connected to a T tube between the bulb and glass tube. The elastic recoil of the compressed air causes the flow between the spurts.

Explain the cause (a) of the increased outflow from the elastic tube with each spurt and (b) of the flow between the spurts. What is the advantage of elastic over rigid tubes when the fluid is forced into them intermittently?

**114. Velocity of the Pulse Wave:** Two levers with their pens writing on the drum in the same perpendicular line are arranged so as to rest at different points on a long piece of elastic rubber tubing; the length of tubing between the levers is three meters. Pressure from a rubber bulb causes an elastic wave to pass over the tube, while the drum is moving at fastest speed. With the drum moving at the same speed, a time tracing is taken in hundredths of a second by a tuning fork.

Observe (1) that the distal lever rises very soon after the proximal one. Note the interval of time between the two tracings, i. e., the time required for the wave to travel three meters, and determines the velocity per second. (2) Observe that the height of the distal wave is much less than the proximal one. (3) Contrast the velocity of the pulse wave with that of the current. Fluids of different colors are in turn



pumped through. Note the time that elapses from the time the fluid enters until it appears at the outflow. (4) That the pulse wave is independent of the flow of fluid is further shown by closing off the outflow. The pulse wave still travels over the entire length of the tube with each increase of pressure from the bulb.

## METHODS OF RECORDING ARTERIAL BLOOD-PRESSURE IN ANIMALS.

**115. (a) Hales' Method:** This consists in connecting an artery of the animal (e. g., carotid artery of a dog) with a long piece of glass tubing that is held in a vertical position. The tube is filled with Ringer's sol. The pressure is measured by the height to which the fluid is forced in the tube when the connection between the artery and tube is completed by removing the clip from the artery. The result is expressed in centimeters of Ringer's sol.; and this may be converted into terms of an equivalent column of water by multiplying the height of the column of Ringer's sol. by its specific gravity; this, in turn, may be converted into terms of a column of blood or of mercury by dividing by their respective specific gravities.

Observe the great height to which the fluid is forced in the tube and the marked fluctuations that occur with the respiratory movements and with each heart beat. Record the result of the experiment and convert it into mm. of mercury.

This means of measuring blood-pressure is mainly of historical interest, it being the first method used. It also impresses one with the great amount of force required to displace the heavy mercury in a mercury manometer.

**116. (b) By the Mercury Manometer:** This consists of a U-shaped piece of glass tubing containing mercury. One limb of the U-shaped tube is connected with the artery by heavy walled tubing (filled with some fluid that delays coagulation of the blood as a 2 per cent solution of sodium citrate). In the distal limb of the manometer a float rests on the surface of the mercury carrying a pen that writes on the drum. The





abscissa or base line is the line made by the pen before the manometer is connected with the artery. The electro-magnet, marking seconds, is arranged so as to write on this line. When the artery is connected with the manometer, the pressure of the blood forces the mercury down in the proximal limb, and up in the distal limb of the manometer, so that the pressure is measured by taking twice the height of the tracing above the abscissa. The mercury manometer is used for recording and measuring *mean arterial pressure*. This is calculated from the tracing by multiplying the distance from a point midway between the top and bottom of the curves to the abscissa by two. The result is expressed in millimeters of mercury.

**117. (c) By the Huerthle Membrane Manometer:** This consists of a small shallow tambour (about 15 mm. in diameter) covered with an elastic membrane. A small disc carrying a recording lever and pen rests on the membrane. A rigid style marks the base line. The chamber of the tambour is connected by heavy tubing with an artery, the tubing being filled with a 2 per cent sol. of sodium citrate to prevent clotting of the blood. The elasticity of the membrane has been calibrated against a mercury manometer so that the height of the pressure tracing may be expressed in millimeters of mercury. The mercury manometer is better suited for recording slight changes in the mean pressure, but the inertia of the mercury prevents an accurate registration of the rapid change in pressure with each heart beat. The membrane manometer follows more accurately the sudden variations in the pressure that take place with each heart beat. For this reason, the membrane manometer is used to determine the *systolic pressure*, i. e., the maximal arterial pressure during systole, and the *pulse pressure*, i. e., the increase in pressure which takes place between diastole and systole. The *diastolic pressure* is that which is maintained in the over-distended arteries between the systoles, i. e., during diastole. The pulse pressure is determined by subtracting the diastolic from the systolic pressure.

Observe the height of the contractions recorded by the membrane manometer as compared with the small waves on the tracing taken by the mercury manometer.





In the experiment which you have seen, record the mean arterial pressure, as determined by the mercury manometer, and find the equivalent of it in a column of water.

Record the systolic, diastolic and pulse pressures, respectively, stating what each one is from what you have seen.

**118. Venous Pressure:** The pressure in the veins is not sufficient to displace a heavy column of mercury, so that a water manometer is used. The manometer and tubing connecting it with the vein are filled with a dilute solution of sodium citrate. A T-shaped cannula is placed in a large vein, like the external jugular of a dog, where two branches of the vein join, so that the lateral pressure may be measured as the blood passes through the vein. (1) Observe and record the *mean venous pressure* in mm. of sodium citrate sol. when the clips are removed from the vein on both sides of the cannula. Convert this into mm. of mercury. (2) Observe and record the changes in venous pressure (a) with inspiration and (b) with expiration. What causes the variations in venous pressure with the different phases of respiration? (3) Observe and record the pressure in the distal end of the vein when the proximal end is occluded by a clip. (4) Observe and record the pressure in the proximal end when the distal end is occluded.

**119. Influence of Saline Infusion on Arterial and Venous Pressure:** Connect the cannula in the vein with a mercury manometer which writes immediately under the mercury manometer recording the arterial pressure. The tubing between the vein and manometer is filled with sodium citrate solution.

After taking a tracing of the normal, Ringer's sol. is injected intravenously at the bodily temperature in such quantity that the volume of blood is increased 50 per cent. Observe the increase of venous pressure without any increase of arterial pressure; also the rapid return of the venous pressure to normal.

After the return to the normal, the volume may be increased 100 or 200 per cent without increasing the arterial pressure. Explain the results.



**120. Influence of Posture on Blood-Pressure:** The blood-pressure is taken in the carotid artery. After the animal has recovered from the anesthetic and regained consciousness, what is the effect upon blood-pressure (in the carotid) of (a) raising the feet? (b) Of raising the head and lowering the feet?

The animal is then etherized again and kept in a condition of surgical anesthesia. (a) What is the effect upon blood-pressure of raising the feet in the state of deep anesthesia? (b) What is the effect of raising the head and lowering the feet? Explain the difference between the two conditions and state why the posture during anesthesia is important.

**121. Effect of Bleeding and of Transfusion upon Blood-Pressure:** What is the effect upon the arterial pressure of removing from one-fourth to one-third of the animal's blood by bleeding from the distal end of the jugular vein?

Observe the change in the pulse pressure as the mean arterial pressure falls. Does the mean pressure improve before transfusion is started? Explain how this is brought about.

The blood is rendered non-coagulable by mixing with sodium citrate in the proportion of 0.25 per cent and injected at the bodily temperature. Observe that the restoration of the volume of blood by injecting the citrated blood causes the arterial pressure to return to normal.

The animal is then bled again to the same extent as the first time and mixed with sodium citrate sol. as before.

Now inject an equivalent amount of Ringer's sol. at the bodily temperature. Observe that the arterial pressure is not completely restored to the normal as Ringer's sol. does not have the same viscosity as blood. The subsequent injection of the citrated blood, or of Ringer's solution made viscid by the addition of a colloid will restore the pressure.

Record the results and explain what becomes of the excess of fluid after saline infusion.

**122. Influence of Respiration upon the Pulmonary Blood-Vessels:** 1. The lung of a frog is exposed through an incision in the flank and arranged so that the vessels may be observed directly with the low power of the microscope. The lung is



distended by positive pressure from within, by means of a cannula tied in the larynx.

Observe that the blood-vessels are narrowed in direct proportion to the distending force of the air within the lung. Extreme distension causes complete blood stasis.

Note that this condition of pulmonary vasoconstriction obtains during the inspiratory phase of artificial respiration in animals; also that a similar condition occurs in certain forms of emphysema of the lungs and explains the hypertrophy and dilatation of the right heart.

2. In another frog the lung is placed in a glass bulb with a narrow opening. The lung is distended by negative pressure in the bulb outside the lung, similar to the negative intrathoracic pressure in mammals.

Observe the great dilatation of the blood-vessels when the lung is distended in this way. Note that this is the condition of the lung and pulmonary vessels during the inspiratory phase of natural respiration. This widening of the pulmonary blood-vessels allows more blood to get through from the right to the left heart during inspiration.

### **123. The Influence of Respiration on the Arterial Pressure:**

The variations in arterial pressure with the different phases of respirations are just the reverse of the changes in the venous pressure. During most of the phase of inspiration the arterial pressure rises, due to a dilated condition of the pulmonary vessels which allows more blood to get through from the right to the left heart. During the greater part of expiration the arterial pressure falls as less blood can get through the constricted pulmonary vessels and return to the left heart.

The respiratory movements are registered by a tambour connected with the trachea. The negative intratracheal pressure shows the time occupied by inspiration, while the positive pressure occurs with expiration. The tambour writes on exactly the same vertical lines as the tracing of the arterial pressure by the mercury manometer.

If the respiratory rate is rapid it may be difficult to determine the exact time of the changes in the blood pressure. When both vagi are cut, or when the trunk of the nerve is frozen so as to





block the nerve impulses and thus prevent them from reaching the respiratory center from the lungs, the respirations become very slow and the changes in arterial pressure caused by the different phases of respiration can be followed. It sometimes happens that there are no respiratory curves on the tracing of the arterial pressure. They usually become more prominent when the air encounters considerable resistance in entering and leaving the lungs.

1. Observe that the *natural respiratory movements* and the large curves on the tracing of the arterial pressure do not exactly coincide. Inspiration begins just before the pressure reaches its minimum, and ends just before the maximum pressure occurs. Expiration follows at once and most of the fall in pressure occurs during this phase of respiration.

2. In *artificial respiration*, as used with animals in the laboratory, the conditions are reversed. Air is forced into the lungs under a positive pressure in inspiration, causing a constriction of the pulmonary vessels and a consequent fall of arterial pressure. During expiration the air escapes with the return of the distended thorax to the normal and the blood pressure in the arteries increases as more blood gets through the lungs. Thus the variations of arterial pressure with artificial respiration are the opposite of those with natural respiration as the mechanism is totally different.

3. *Positive ventilation* of the lungs or inflation with compressed air forced into the trachea, under sufficient pressure to produce moderate distension of the thorax, causes inhibition of the respiration and a fall of arterial blood pressure due to the constriction of the pulmonary blood vessels. The extent to which the arterial pressure falls depends upon the amount of intrapulmonary pressure exerted by the air in distending the lungs. The arterial pressure may be reduced to one-third or one-fourth of the normal by producing considerable distension of the lungs and thorax. The vascular change (constriction) seen in the frog's lung from inflation by means of a cannula in the trachea explains the tremendous fall of arterial pressure produced in the dog by the same procedure.

4. *Negative ventilation* of the lungs may be produced by causing the animal to breathe rarified air. The tracheal cannula



is connected with a large vessel or tank containing a considerable volume of air under negative pressure of  $-15$  to  $-30$  mm. Hg. Observe that the breathing of air under less than the ordinary atmospheric pressure causes a rise of arterial pressure due to dilatation of the pulmonary vessels. Such change in arterial pressure does not occur in going from the sea-level with high atmospheric pressure to the low barometric pressure of a high altitude, because the peripheral vessels of the pulmonary and those of the systemic circulation are subject to the same change and one balances the other.

It is clear that negative intrapulmonary air pressure causes the vessels of the lungs to dilate in the same way that the vessels of a frog's lung can be seen to be dilated when the lung is distended in a glass chamber by a negative pressure outside the lung.

(a) Explain the cause of the rise of arterial pressure during inspiration, and the fall with the expiratory phase of natural respiration.

(b) Explain the respiratory changes of arterial pressure during artificial respiration as it is used in animals.

(c) Describe the effect upon arterial pressure of breathing air from a pneumatic cabinet under positive and negative pressure, respectively, without exposure of the exterior of the body to the same atmospheric pressure.

**124. Influence of Asphyxia on Arterial Blood Pressure:** When the air is prevented from entering the lungs so that the interchange of gases between the air and blood can not take place, the respiratory movements are at first very rapid (hypernea). The vigorous and struggling respiratory movements cause enormous respiratory curves on the tracing of the arterial pressure. Gradually the movements become less rapid but more labored (dyspnea) with active expiratory effort. This stage of excitement may end in convulsive movements.

During this stage there is pronounced cardiac inhibition from stimulation of the cardio-inhibitory center. The blood pressure steadily rises, in spite of the cardiac inhibition, from direct stimulation of the vaso-constrictor center by the venosity of the blood. A general convulsion also causes rise of blood-pressure.



Sometimes the cardiac inhibition is sufficient to offset the vasoconstriction. When that is the case, the administration of atropine causes paralysis of the cardio-inhibitory cells and the rise of pressure from stimulation of the vaso-motor center can then be demonstrated.

During the third stage, or that of collapse, the blood-pressure is low from paralysis of the vasomotor center; the respirations have ceased or only occur at long and irregular intervals; the heart continues for a considerable time after respirations cease. The spontaneous respirations can be re-established and the blood-pressure can be restored to normal by lowering the head and using artificial respiration.

### NERVOUS CONTROL OF THE BLOOD VESSELS.

**125 The Circulation in the Web of the Frog's Foot:** Observe (1) the velocity pulse in the arterioles; (2) the comparatively slow current, without pulsations, in the small capillaries; (3) the venules are larger vessels with swifter current but free from pulsations. Observe that the corpuscles move much slower at the periphery than in the middle of the stream. From this fact, what would you consider the chief cause of the resistance encountered by the blood?

Why is the velocity less in the capillaries than in the arteries and veins?

Explain the cause of the continuous flow, without pulsations, in the capillaries.

**126. Efferent Vasomotor Fibers in the Sciatic:** In a frog that has been lightly curarized the sciatic nerve is cut on one side. The webs of both feet are pinned out on a cork plate and placed on the stages of two microscopes placed side by side. In this way comparison can be made with the normal. (1) Observe the dilated blood-vessels and increased flow of blood which results from section of the nerve. Explain the cause of this condition and tell how *vasomotor tone* is maintained normally. (2) Electrodes are placed under the peripheral end of the sciatic and the nerve is stimulated with a tetanizing current. Constriction in the individual vessels can not be seen, but the vascularity is





greatly decreased during stimulation and the flow is lessened or stopped in the capillaries. What change takes place in the arterioles during stimulation of the nerve? Does vasoconstriction occur in the arterioles or capillaries? Note that the vasodilatation soon returns after the stimulation ceases.

**127. Vasomotor Fibers of the Cervical Sympathetic:** The ears of a rabbit are shaved and the cervical sympathetic is cut on one side. (1) What is the condition of the larger blood-vessels of the ear on that side? How does the pink color of the ear between the blood-vessels compare with that of the other side? Explain the cause of this increased vascularity. (2) The peripheral end of the sympathetic is stimulated with a tetanizing current. What is the effect upon the blood-vessels of the ear of that side? (Comparison should be made with the normal side in each case.)

**128. The Depressor Nerve in the Rabbit:** In the rabbit this is a branch of the vagus, found in the sheath of the carotid with the vagus and sympathetic, and consists of afferent vasodilator or depressor fibres. (1) Observe that stimulation of this nerve, with the vagi intact, causes a fall of pressure with pronounced cardiac inhibition. What conditions result from the stimulation of the nerve which may explain this fall of pressure? (2) The vagi are cut. Observe that stimulation of the nerve now causes just as great a fall of pressure without any cardiac inhibition. Explain the cause of the lowered pressure in this case. (3) Observe that section of the nerve does not cause any change in the blood-pressure. (4) Observe that stimulation of the peripheral (cardiac) end has no effect, while stimulation of the central end causes as great a fall of pressure as was obtained when the unsevered nerve was stimulated.

Is the vasodilatation which results from stimulation of the depressor nerve a local one, due to stimulation of efferent vasodilator fibers, or is it a general vasodilatation produced reflexly by stimulation of afferent vasodilator (depressor) fibers? Under what conditions would these nerve fibers be stimulated normally? Make a protocol of the experiment to show these points.





**129. Vasomotor Functions of a Mixed Spinal Nerve, e. g., the Sciatic:** (1) What is the effect upon general arterial blood-pressure (in the dog) of stimulating the sciatic nerve intact? Explain what conditions may cause the rise of pressure. (2) Observe that section of the nerve has no effect upon mean arterial pressure; neither does stimulation of the peripheral end, although there is a local vasoconstriction, as was seen in the frog's foot. Explain why this local vasoconstriction fails to influence the mean arterial pressure. (3) Observe that stimulation of the central end produces just as great a rise of pressure as was obtained at first. After section of the great splanchnic nerve there is little or no rise of pressure from stimulation of the central end of the sciatic. Explain how stimulation of the central end causes a rise of pressure.

**130. Vasomotor Fibers of the Splanchnic Nerve:** The great (left) splanchnic nerve is exposed just above the diaphragm by resecting a portion of the lower ribs in a dog. (1) What is the effect upon the blood-pressure and heart rate of stimulating the trunk of the nerve with both vagi intact? Is the cardiac inhibition sufficient to prevent a rise of pressure? (2) Observe that section of the great splanchnic in the dog does not cause a fall of pressure. (In the rabbit the pressure is lowered by cutting this nerve.) (3) Observe that stimulation of the central end of the cut splanchnic fails to cause a rise of pressure, while stimulation of the peripheral end causes as great an increase as before. Compare this with the stimulation of the sciatic nerve in the last experiment and explain the difference in the results obtained. (4) Both vagi are cut to sever the efferent cardio-inhibitory nerve fibers going to the heart. Observe that reflex cardiac inhibition can not be obtained by stimulating either end of the cut splanchnic after section of both vagi. (5) When the peripheral end of the splanchnic is stimulated with the same strength of current as before, observe that the rise of pressure is greater after section of the vagi than when they are intact, as there is no inhibition of the heart.

Make a protocol of this experiment and write a brief summary of the vasomotor function of the splanchnic nerve.

**131. Vasomotor Changes Produced by Drugs:** (1) Adrenalin



is injected intravenously (10-20 c. c. of 1-20,000 Sol.). Observe the pronounced rise of pressure from the vasoconstriction which passes off in a few minutes. With the vagi intact, adrenalin often causes such a degree of cardiac inhibition that there is comparatively little increase in the mean pressure. Section of both vagi prevents the cardiac inhibition to a certain extent, but not entirely, as the drug appears to act upon the inhibitory nerve cells within the heart as well as the neurones in the medulla. If cardiac inhibition persists after section of the vagi, it may be removed by a full dose of atropine.

Observe that the rise of pressure from adrenalin is very great when the vasoconstriction is not offset by cardiac inhibition. The pressure may amount to double or treble the average height.

(2) The inhalation of amyl nitrite causes a pronounced fall of pressure due to vasodilatation. Observe that the effect soon passes off.

(3) The influence of partial asphyxia upon the blood-pressure may be shown before and after cutting the spinal cord. The fact that rise of pressure from vasoconstriction only occurs while the spinal cord is intact shows that the vasomotor center is stimulated by the venosity of the blood. The best results are obtained when the first observation is made before the great splanchnic nerve is cut.

The spinal cord is cut in the upper cervical region and artificial respiration is maintained. Observe the great fall of blood-pressure which follows section of the spinal cord and explain the cause of it.

Now adrenalin is given again in exactly the same dose as before. Observe that adrenalin produces relatively as great a rise of pressure as before. Of course, the pressure does not reach the same absolute elevation as in the normal condition. From this experiment what would you conclude as to the way vasoconstriction is produced by adrenalin?

**132. Traube-Hering Curves:** A dog that has had both vagi severed to remove the nervous control of the cardio-inhibitory centers upon the heart, has a tube tied in the trachea for carrying on artificial respiration. Curare is then injected into the vein slowly and cautiously until voluntary movements and



the respiratory movements cease. Care should be exercised to give just enough to paralyze the motor nerves, but no more, as an excess causes vasomotor paralysis. Artificial respiration is then established for a few minutes. The respiratory curves on the arterial pressure, if they now occur, show a fall during inspiration and a rise during expiration, just the reverse of those seen in natural respiration. The tube through which the lungs are inflated is then detached from the tracheal tube for short periods of time while the drum is revolving. It will be seen that slow, rhythmical curves occur on the arterial pressure tracing although there is no respiratory or voluntary movements. These are known as Traube-Hering curves and are due to rhythmical activity of the dominating vasoconstrictor center. One of these occupies as much time as several respiratory curves. Sometimes curves occupies as much time as several respiratory curves. Sometimes they may be seen when respiration is going on.

**133. Circulation Time:** This is determined (for class demonstration) by injecting 5 to 8 c. c. of a saturated solution of medicinal methylene blue into the veins at different points of the circulation, and, with a stop watch, noting the time that is required for it to appear at other points. Methyl blue is changed to a colorless substance in a few minutes in the blood so that the observations may be repeated a number of times.

1. Observe the time that intervenes between the injection of methyl blue into the external jugular vein and its appearance in the carotid artery. This gives the time required for a circuit of the lesser circulation, and, in small dogs, it is not more than three or four seconds.

2. Observe the time required for the pigment to appear in the femoral artery after an injection into the jugular vein.

3. Observe the time which intervenes between the injection of the pigment into the femoral vein and its appearance in the femoral artery. Note that this is very little more (about two seconds) than the time required for the lesser circulation.

4. If the large branch of the femoral artery (*profunda femoris*) is large enough for the introduction of a cannula, an injection of methylene blue may be made into the femoral artery without interfering with the natural flow of blood through its





branches. Observe the time that intervenes between the injection of methylene blue and the appearance of the blue color in the femoral artery of the opposite side. This represents the complete circulation time, i. e., the time of the lesser circulation plus the longest loop of the greater circulation.

Record the results of two observations in each case.

**134. Measurement of the Velocity of the Blood Stream by Ludwig's Stromuhr:** This is a U-shaped tube with bulbous enlargements of definite capacity; the ends of the tube are attached to the upper of two disks, so that the bulbs may be reversed quickly without any leakage; two tubes from the lower disk enable the bulbs to be put in communication with both ends of a severed artery; these tubes are fitted with cannulas of different sizes, so that the instrument is connected with the artery by cannulas having the same lumen as the artery.

The instrument measures the volume of blood that passes through a vessel of definite size in a given unit of time (one second). The velocity is determined by dividing this volume of blood by the area of the transverse section.

A small dog is used and the coagulability of the blood destroyed by the intravenous injection of 0.001 gram. of Hirudin for every 8 c.c. of blood in the body.

The proximal bulb (i. e., toward the heart) is filled with oil; the distal one with defibrinated blood. When the clips are removed from the artery the blood from the central end of the artery rises under the oil and displaces the defibrinated blood from the distal bulb into the peripheral end of the artery. When the oil has all been displaced to a certain mark on the distal bulb, the bulbs are quickly reversed and the one containing oil is again placed in communication with the central end of the artery.

Seconds are registered on a revolving drum and an electro-magnet connected with the disk of the stromuhr, writes on the same vertical line as the second marker. Each time the bulbs are reversed, electrical contact is made by the disk and marked on the drum by the electro-magnet. The bulbs are reversed, as often as the oil is displaced, for one or two minutes, and an average taken.



Having determined the average amount of blood that escapes in one second, and the transverse section of the artery, the velocity is found by dividing the former by the latter. For instance, if 1 c.c., or 1000 c. mm., escapes per second, and the sectional area of the artery is 3.14 sq. mm., then the velocity is 318 mm. per second.

**135. Velocity in the Capillaries:** This may be determined by direct observation of the flow in the web of the frog's foot, using a micrometer eyepiece and an electro-magnet to register the time of each observation on a drum moving at fast speed. A time-marker should register tenths of a second just beneath the electro-magnet.

The eyepiece is arranged so that the micrometer scale coincides with the stream in as long a stretch of a capillary as possible. The vision is fixed on some one corpuscle, and with the hand on the key the time required for the corpuscle to travel from one part of the scale to another is indicated by the electro-magnet. The amplification being known, it is an easy matter to calculate the distance traveled per second.

The average of a number of observations should be taken. In this experiment much depends upon the training and care of the observer. From a comparison of the velocity of the blood in the large arteries with the velocity in the capillaries, what would you conclude as to the combined sectional area of the capillaries compared with that of the arteries?

**136. The Volume Pulse:** This may be determined (a) by using two *small plethysmographs* or glass tubes, into which two fingers are inserted with a rubber collar fitting tightly about them at the mouth of the tube. The rubber should not be tight enough to interfere with the venous circulation.

The plethysmographs are then connected by means of a Y-tube and rubber tubing with a delicate recording tambour, with a T-tube interposed between the two. When everything is adjusted a spring clamp is placed on the side arm of the T. With each heart beat there is an increase in the volume of the finger and the pulsations are registered by the tambour.

The drum must be smoked very lightly and the lever of the tambour must write with the least friction possible. By care-



fully counterposing the lever of the tambour, good tracings of the volume pulse can be obtained, showing a distinct dicrotic notch.

(b) The *large plethysmograph* for enclosing the hand and forearm gives much larger pulsations than those obtained from the fingers. The hand is held in a special glass cylinder; the hand and forearm are surrounded by water at the temperature of the body; with each heart beat the water is displaced by the increase in the volume of the part, giving pulsations with the dicrotic notch in the usual position. These may be recorded by a tambour or by registering the changing level of the fluid as it is displaced from the plethysmograph.

This instrument is used for determining the changes in the peripheral blood-vessels. With dilatation of the surface vessels, the volume of the hand increases greatly, while the opposite change occurs with vasoconstriction.

(c) The *oncometer*: This is a special plethysmograph for measuring the volume of the kidney or spleen and the changes that take place with varying conditions of the blood-vessels. It consists of a metal box, made in two halves, each lined with delicate peritoneal membrane. The kidney is encased without interfering with its circulation or the outflow of urine; the space between the kidney and the box is completely filled with a light oil at the temperature of the body; this communicates with a piston recorder or an oncograph so that the increase in the volume of the kidney with each heart beat may be recorded on a revolving drum. The pulsations are very pronounced and show the dicrotic notch as distinctly as the tracings of the arterial pulse.

Enormous changes in the volume of the kidney may be shown by producing general vasodilatation by the inhalation of amyl nitrite, and by producing vasoconstriction by the injection of adrenalin. In the former case the kidney expands and the oncograph tracing rises as the arterial pressure falls; in the latter, the volume decreases and the plethysmographic tracing falls as the arterial pressure rises.

The same changes may be shown by cutting the splanchnic nerve of the same side to produce vasodilatation and by stimu-





lating the peripheral end to cause vasoconstriction. The infusion of Ringer's Sol. also causes the kidney to swell by increasing the capillary pressure, although the arterial pressure is not changed.

**137. Arterial Pressure in the Frog:** The frogs have been curarized and do not require pithing, except to destroy the brain without destroying the medulla. Pin the frog out on the frog plate; cut through the skin in the usual way from the pelvis to the jaw. Now tie the epigastric vein near the pelvis by making a nick through the abdominal wall on each side of it, and passing a ligature beneath it. Expose the heart in the usual way, and tie the frenum. *Be careful to avoid hemorrhage.*

Clear the two aortæ. Notice which is the larger (usually the left); pass a ligature beneath it and tie as far away from the heart as possible. Fill the cannula and tube with sat. sol. of sodium bicarbonate and close the end of the tube with a piece of glass rod. Place a small clip on the left aorta close to the bifurcation, and tie a second ligature loosely about the vessel between the clip and the first ligature. Make a small opening in the vessel between the first ligature and the clip, and insert the point of the cannula into it; let your partner fasten it in place by tying the second ligature about its neck. Fasten the frog plate to the stand at a little higher level than the manometer. See that the proximal limb of the manometer is filled with soda solution to the exclusion of all air bubbles. Arrange the electro-magnet so that its pen writes on the same horizontal line as the pen of the manometer before the rubber tube of the cannula is attached to it. Connect the electro-magnet with the time circuit to mark seconds. The line made by the electro-magnet will now mark the level of atmospheric pressure in the manometer, or the "*abscissa*" of the blood-pressure tracing.

Now pinch the rubber tube behind the glass rod and remove it; fill the end of the tube with soda solution; attach it to the manometer before releasing the constricting fingers. Note that as soon as the tube is released the mercury in the proximal limb of the manometer falls, and rises correspondingly in the distal end, and that with each beat of the heart the mercury rises and falls.





Take a normal tracing two or three inches in length with the drum at very slow speed.

**138. Effect of Cardiac Inhibition on Blood-Pressure:** Arrange the inductorium for the tetanizing current and attach the hand electrodes to the secondary. Find the strength of current that will cause marked inhibition when applied to the crescent. Set the drum going again at very slow speed; raise the heart gently and stimulate the crescent, your partner marking on the drum the beginning and end of the stimulation. Do not stop the drum until the pressure and rate have returned to normal. What effect has inhibition of the heart on blood-pressure?

**139. Effect of Stimulating a Sensory Nerve on Blood-Pressure:** After waiting a few moments, set the drum going at the same speed as before, and with the hand electrodes, stimulate the brachial plexus (the single large nerve trunk going to the fore leg) on the side opposite to that in which the cannula is tied in the aorta. Observe the rise in pressure. How is this produced?

*Effect of Destroying the Spinal Cord:* Carefully turn the frog on its side and pith it; then destroy the cord by pushing the pin into the spinal canal. Return the frog to its original position and take a tracing. Observe that there is a marked fall in pressure. Why?

After your tracings have been varnished and dried, measure the blood-pressure in each one. Mark the mean between the top and bottom of the curves and multiply the distance (in mm.) between this point and the abscissa by two. Record the results in mm. of Hg in a protocol of the experiment.

**140. Perfusion of the Blood-vessels:** Pith a frog and destroy its brain by passing the pin anteriorly, but do not destroy the cord. Expose the heart in the usual way. Grasp the ventricle with forceps and with the points of scissors make a nick in the sinus venosus. Let the heart bleed freely for a few minutes, removing the blood and clots with moist cotton. Fill the T-cannula and tubing with Ringer's solution; close the short lateral tube with a clip, and the long tube with a piece of glass rod. *Be sure to exclude all air bubbles from the cannula and tubing.*



Place a soft moist ligature about the auricles so that it can be tied over the bulbus arteriosus. Hold the ventricle with forceps and with the scissors make a small nick or V-shaped opening at the base of the ventricle or in the bulbous arteriosus close to its origin. Insert the cannula into the bulbus arteriosus and compress the rubber tubing gently to see that the bulbous arteriosus and aorta are distended during the compression. This makes sure that the cannula has not passed back into auricle through the tricuspid orifice.

Let your partner fasten the cannula in position by tying the ligature about its neck and tie the ends of this ligature about the lateral arm of the cannula to keep it from pulling out. Hook a wire through the frog's nose, and hang it by the wire to the funnel ring. Remove the rod from the rubber tubing and connect it with the thistle-tube funnel. Fill the funnel with Ringer's solution and open the lateral tube of the cannula to allow any air bubbles that may be in the tubing to escape. The fluid, entering the aorta, makes the complete circuit of the frog's vessels, and returns to the sinus venosus, where it escapes, and, running down the frog's legs, drops from its toes into a pan placed beneath them. Let the perfusion continue until the fluid dropping from the toes is fairly free from blood.

1. Now count the number of drops falling from the toes in two periods of two minutes each, and record the results in a protocol as *the normal*.

2. Empty the funnel through the side tube, and refill it with a solution of adrenalin in Ringer's sol. Let the fluid perfuse for a few minutes (until the flow is markedly diminished) and count the number of drops in two periods of two minutes each. Record your results as before.

3. Empty the funnel, and refill it with a solution of amyl nitrite in Ringer's sol. Let the fluid perfuse for a few minutes (until the number of drops from the toes show marked increase). Count the number of drops in two periods of two minutes each. Record the results.

4. Empty the funnel and perfuse again with Ringer's sol. Count the number of drops for two periods of two minutes each for the *second normal*.

5. Now destroy the spinal cord and count the number of



drops for two periods of two minutes each. Record the results.

6. Perfuse again with adrenalin solution and record the number of drops in two periods of two minutes each.

7. Empty the funnel and refill it with amyl nitrite solution. Count the number of drops in two periods of two minutes each. The effects of these drugs are shown here not particularly to demonstrate their action, but to show how constriction and dilatation of the blood-vessels influence the flow of fluids through them.

Observe that adrenalin causes a marked decrease in the flow by constricting the vessels, both before and after destroying the spinal cord. Does it produce this effect by its action on the nerve centers or by direct action upon the vessel walls? Observe that amyl nitrite causes a decided increase in the flow by dilating the blood-vessels, both before and after destroying the cord. How does it produce the dilatation? Observe that destroying the spinal cord greatly increases the flow of Ringer's sol. through the vessels, because destruction of the vasomotor centers in the cord removes vasomotor tone.

### ARTERIAL PRESSURE IN MAN.

**141. The Sphygmomanometer:** A number of instruments have been devised in recent years for measuring blood-pressure in man. The principle is practically the same in a number of them. The one designed by Erlanger gives accurate results by the graphic method.

On the inside of a rigid leather armlet or cuff is a broad, flat rubber bag which can be inflated with air so as to compress the brachial artery. The armlet is buckled about the arm so that the rubber bag comes over the brachial artery; the rubber bag is connected, by means of a three-way stop cock, (a) with a rubber bulb for inflating the bag, (b) with a mercury manometer for registering the pressure and (c) with a thick-walled rubber bulb enclosed in a glass chamber. The pulsations in this bulb are transmitted by the air in the glass chamber to a tambour; the lever of the tambour magnifies and records the pulsations on a revolving drum.





Owing to the compressibility and elasticity of air, the pressure within the arm-piece is the same as in the other parts of the apparatus, and it is measured by the mercury manometer.

The pressure which is just sufficient to obliterate the radial pulse corresponds to the *maximal* or *systolic pressure* in the artery. With most instruments this point is determined by feeling the radial pulse at the wrist. With the Erlanger apparatus this point may be seen on the tracing by the *sudden increase in the height of the pulsations* when the pressure is lowered, after having been above the pressure required to obliterate the artery. This point shows the pressure at which the blood just begins to get through when the compression is lessened, and is usually from 5 to 10 mm. of mercury lower than the point at which the radial pulse disappears at the wrist.

The *diastolic pressure*, or the pressure between the beats of the heart, corresponds to the pressure at which the greatest pulsations occur. It has been found by experiment that the greatest lateral expansion of the artery with each heart beat, occurs when the pressure on the outside of the artery is just equal to the diastolic pressure of the blood within the artery.

With the Janeway and Stanton sphygmomanometers (which consists of a broad arm-piece and a mercury manometer with narrow lumen) this point is determined by observing the pressure at which the greatest pulsations of the mercury occur. With the Erlanger apparatus the *last high waves on the tracing*, as the air is allowed to escape slowly, mark the *diastolic pressure*.

The *mean arterial* pressure is determined by finding the mean between the systolic and diastolic pressure.

Each student will in turn determine the blood pressure in man (a) with the Erlanger sphygmomanometer; (b) with a modified Erlanger apparatus; (c) with a modified Stanton or Janeway apparatus; (d) with the Faught sphygmomanometer, and (e) by the auscultatory method.

The individual whose pressure is to be determined is seated near the table with the forearm resting on it, at the same level as the heart. The arm-piece is fastened snugly, but not too tightly, about the arm just above the elbow. Care should be taken that the rubber bag is over the line of the brachial



artery. The arm-piece is inflated until the pulse at the wrist is just obliterated. The pressure at this point should be noted.

The side tube is now opened and air allowed to escape through a fine capillary tube until the pulse is perceptible at the wrist. The pressure at this point is also noted and the mean between the two may be taken as the systolic pressure. The observation should be repeated several times until uniform results are obtained.

Now inflate the arm-piece until the pressure in the arm-piece is above the systolic pressure; allow the air to escape slowly through the side opening, watching carefully the pulsations in the mercury manometer; observe the pressure at which the highest pulsations occur. This is the diastolic pressure. Repeat the observation several times.

**Auscultation Method:** Inflate the pneumatic bag as before until the artery is obliterated. A stethoscope is applied over the artery below the cuff and the pressure within the bag is allowed to fall gradually. A distinct sound or sharp thump is heard at the point at which the blood gets past the compression and the circulation commences. This marks the systolic pressure.

The sound undergoes a number of changes as the pressure in the pneumatic bag is lowered. Just before it disappears it change from a loud to a dull, muffled sound. This corresponds to the diastolic pressure. The sound completely disappears when the pressure in the bag falls a few millimeters below the diastolic pressure.

Repeat these observations several times until uniform results are obtained. Record the results by the different methods and compare them.

Erlanger gives the average systolic pressure at 110 mm. and the average diastolic pressure as 65 mm. for young adults under 25 years of age. The average obtained in this laboratory upon fifty healthy medical students is 127 mm. Hg for the systolic and 85 mm. Hg for the diastolic pressure. With the Janeway apparatus the figures are usually from 5 to 10 mm. higher than those obtained with Erlanger's sphygmomanometer.

**142. The Blood-Pressure in the Capillaries:** This can not be measured directly, but is determined by the pressure re-



quired to obliterate the capillaries in compressing a thin layer of soft vascular tissue.

(a) See that the capillaries of the hand are filled (by holding it down or by placing it in warm water for a short time). Place the little glass square of the special weight pan on the skin just back of the finger nail, holding the hand at the level of the heart. This glass is  $1\frac{1}{2}$  cm. square, and therefore has an area of  $1\frac{1}{4}$  sq. cm. Add weights to the pan until the pink color of the skin disappears. This marks the point at which the capillaries are obliterated. Add the weight of the pan to the weights in it and divide by 1.359. The result will express the pressure in mm. of mercury as a vertical column of mercury 1 mm. high and  $1\frac{1}{4}$  sq. cm. in section exerts a pressure of 1.359 grams. Record the result of your observations.

(b) Place the finger on the pneumatic cushion made by a finger cot in a curved metal support. Inflate the finger cot until it presses the finger against the glass across the top of the frame with sufficient force to obliterate the blood vessels. The pressure is indicated by a mercury manometer connected with a T-tube between the inflating bulb and the finger cot.

**143. Tracing of the Pulse Wave:** Smoke the drum lightly and arrange the recording tambour to write with the least friction possible. Connect the tambour with a thistle tube by heavy tubing, with a T-tube interposed between the two. See that the side arm of the T-tube is open. Use the thistle tube as a receiving tambour (without a membrane) by pressing backward and inward over the carotid artery, just in front of the sterno-cleido-mastoid muscle, about the level of the thyroid cartilage. (The point of the best pulsation can readily be determined by first pressing with the finger.) When all is ready have the assistant close the side arm of the T-tube with a spring pinchcock and set the drum at slow speed. If good pulsations are not obtained at first, the pressure with the thistle tube should be varied until good pulsations are registered on the drum. The arm and hand holding the thistle tube should be held steady, resting on the table in a comfortable position.

Take one tracing with the drum at such speed that each wave occupies about 1 cm. on the tracing, and then take an-





other with the drum at fast speed. With the electro-magnet record seconds under each tracing with the drum at the same speed as before.

Observe (a) the percussion cap; (b) the dicrotic notch and dicrotic wave; (c) the predicrotic wave which may not be constant.

**144. Sphygmogram with the Dudgeon Sphygmograph:** With the finger first determine the position at the wrist where the pulsation of your partner's radial artery is greatest. Adjust the Dudgeon sphygmograph so that the pad comes exactly over this point and hold the instrument securely by the tapes passed about the wrist. See that the clock work is wound; vary the pressure of the spring and pad over the artery until the greatest excursions of the recording pen are obtained; then take a tracing. Varnish the tracing and paste it in the book with a brief description of each part.

**145. Cardiogram:** Smoke the drum lightly and arrange a recording tambour as before. Connect it with a small funnel by rubber tubing with a T-tube between the two.

Adjust the small funnel which acts as a receiving tambour over the apex beat or cardiac impulse and hold it steady while your partner takes the tracing. The best results are obtained by leaning forward and to the left.

Take one tracing with the drum at moderate speed and another with the drum at fast speed. With the electro-magnet record seconds beneath each tracing with the drum at the same speed as before.

Observe the small wave which precedes the main wave. The former corresponds to the systole of the auricles and is caused by the complete filing of the ventricles at that phase of the heart cycle; the latter marks the systole of the ventricles and is due to the hardening of the heart muscle which comes in closer apposition with the chest wall during contraction.

The best pulsations are obtained between respirations by holding the breath after a deep expiration. The respiratory movements of the chest and the expansion of the lung interfere with uniformity in the tracing.

If good cardiograms can not be obtained at first, a little active exercise will cause a stronger cardiac impulse.





**METABOLISM.****ABSORPTION FROM THE ALIMENTARY CANAL.**

**146. Seat of Absorption:** A fasting dog or cat is anesthetized and a small opening is made in the abdominal wall to expose the stomach and small intestine. If the intestine contains any food it is emptied by stripping between the fingers. Two ligatures are tied around the intestine about three feet apart; ligatures are also tied about the cardiac and pyloric orifices of the empty stomach.

The same amount of water is then injected into the stomach and into the loop of the intestine (50 c.c. for a cat; 75-100 c.c. for a dog). The injection is made by means of a hypodermic needle passed obliquely through the wall. Some blood serum may be injected into another loop of the small intestine.

After 60 to 75 minutes the animal is killed and the water that remains in the stomach and in the loop of the intestine is collected separately and measured. Observe (1) that relatively little of the water has been absorbed from the stomach, although its wall is as vascular as that of the intestine; (2) that most or all of the water has been absorbed from the intestine; (3) that blood serum is absorbed from the intestine although it has the same osmotic pressure as the blood and the capillary blood pressure is much greater than the pressure in the lumen of the intestine. Record the results.

**147. Absorption of Colloids and Crystalloids from the Intestine:** A fasting dog is anesthetized and the small intestine is exposed. It is emptied of any food that may remain by stripping the jejunum and ileum between the fingers from above downward. The intestine is then divided into four separate loops, each about 15 inches long, with ligatures tied lightly about the upper and lower ends. Each loop has two ligatures, with 1 or 2 cm. of intestine intervening between the loops, so that each one can be removed separately later and its contents collected for measurement.

With a large syringe and a fine hypodermic needle 20-25 c.c.



the following solutions are injected into the lumen of the different loops from above downward:

- (a) 10 per cent sol. of egg white.
- (b) 2 per cent sol. of commercial peptone.
- (c) 2 per cent sol. of dextrose.
- (d) 2 per cent sol. of potassium iodide or a 0.25 per cent sol. of sodium sulphate.

The intestine is carefully returned to the abdominal cavity with as little injury as possible and the blood supply of the intestine is not disturbed.

At the end of one and one-half hours the animal is killed and the loop removed. The fluid in each loop is collected separately and measured.

Observe (1) that there has been complete absorption of the peptone; (2) that a large part but not all of the egg albumin solution has been absorbed; (3) that the loop into which dextrose had been injected is entirely empty as there has been complete absorption; (4) that the potassium iodide or sodium sulphate shows comparatively little absorption, the greater part remaining in the loop into which it was injected, although it is as diffusible as dextrose.

Quantitatively the amount of albumin, potassium iodide, or sulphate remaining unabsorbed in the intestine can be determined. It corresponds practically to the proportion of the solution which remained in the loop unabsorbed.

Record the result and explain the absorption of the colloidal and non-diffusible egg albumin.

Explain why one crystalloid (dextrose) is completely absorbed and the other only slightly.

Can absorption from the living intestine be explained by known physical laws?

How does absorption from the intestine differ from absorption from the tissues and from the serous cavities of the body.

**148. Absorption from the Large Intestine:** This may be shown in the dog by the rectal injection of 75 per cent ether in oil after the colon has been emptied. When it is retained there is sufficient absorption to produce narcosis.

Absorption of ether vapor takes place to a sufficient extent to



produce anesthesia in a rabbit when a catheter passed into the rectum is connected with a flask containing ether and immersed in a basin of warm water to promote vaporization.

**149. Glycogen in the Liver:** A rabbit is fed upon a liberal allowance of starchy food for a day before the experiment. The animal is killed by bleeding and the liver quickly removed. It is chopped into small pieces; a part of the liver is weighed and quickly thrown into a definite amount of boiling water made slightly acid by the addition of a few drops of acetic acid. This kills the liver cells, coagulates the proteids and destroys any enzymes which might convert the glycogen into dextrose.

After boiling for a time, the liver is ground up in a mortar with washed sand, boiled again and filtered while hot. The nitrogenous bodies which have not been coagulated by heat are then removed from the filtrate by Brucke's method (i. e., adding conc.  $\text{HCl}$  and potassio-mercuric iodide sol. alternately until no more precipitate forms). The precipitate is filtered off and the filtrate is comparatively a pure sol. of glycogen. Distilled water is added to the filtrate to bring it up to a definite volume and restore the water lost by boiling.

(1) Note the opalescence of the solution which is characteristic of glycogen. (2) Observe the deep reddish-brown color from the addition of a few drops of a weak iodine solution. (3) The glycogen may be precipitated by adding an excess of alcohol.

Pflüger's method of determining the amount of glycogen in tissues insures more complete extraction than Brucke's method, but it requires a longer time and for that reason can not be used for the purposes of the present and subsequent experiments. It is based on the complete digestion and solution of the tissue by boiling in 60 per cent  $\text{KOH}$  for three hours. The glycogen is then precipitated by adding alcohol in such amount that all of the glycogen but none of the protein matter is precipitated. By repeated washings and precipitation the glycogen is obtained in pure state.

**150. Glycogen in Muscles:** An equal quantity (by weight) of the voluntary muscles of the same rabbit is chopped up and extracted in the same way in the same volume of water. After removal of the nitrogenous bodies, water is added to restore that





lost by boiling and bring the solution up to a definite volume as before. Comparison may be made between the two extracts as to the amount of glycogen present.

Note that muscles contain glycogen and the extracts respond to the tests given above. How does it compare in amount with the quantity of glycogen in the extract of the liver? Record the result.

**151. Disappearance of Glycogen in the Liver:** A part of the rabbit's liver is kept moist in the incubator at bodily temperature for an hour or more. A definite amount, by weight, is then extracted in a proportionate volume of water in the same way as before. This should be compared with the extract of the first portion of liver.

Observe that the amount of glycogen is much less. This may be determined (1) by the degree of opalescence; (2) by the depth of color with iodine in comparative tests; (3) by the precipitate with alcohol. Record the results.

Observe also that the amount of sugar in the second portion of liver is greater than that in the first portion. This may be determined by the amount of precipitate formed by boiling a definite amount of diluted Fehling's sol. with the same amounts of the liver extracts in comparative tests. Record the result.

From what you have seen, write a brief account of the relative amount of glycogen stored in the liver and muscles and the change that takes place in it after death.

**152. Ability of the Blood to Handle Dextrose; Glycosuria from Hyperglycemia:** A dog is prepared for making intravenous injections by the jugular vein; the urine is collected from the ureters. Some normal urine is collected and examined to see that it is free from sugar.

The amount of blood, and the quantity of dextrose in solution in the blood, are calculated from the weight of the animal. This may be based upon the average normal amount (0.1 per cent) or the maximum amount normally present (0.15 per cent).

A sufficient amount of dextrose is then injected to increase the quantity of sugar in the blood first by 50 per cent, and later by 100 per cent. The urine is then collected for periods of five minutes each after each injection. Note that the urine remains



free from sugar until the percentage of sugar in the blood exceeds 0.2 per cent, or in exceptional instances 0.3 per cent.

Observe that glycosuria does not occur while there is less than 0.2 per cent of dextrose in the blood. Make a protocol and record the result of the experiment.

**153. Effect of Injecting Maltose, Blood Serum and Foreign Proteins:** 1. Another dog is prepared in the same way. The normal urine is collected and examined to be sure that it is free from sugar and albumin. A small amount of maltose (0.1 per cent of the blood or less) is injected intravenously and the urine collected. Observe that maltosuria occurs within a few minutes. (a) Can maltose be utilized when injected intravenously? (b) What change takes place in maltose as it is absorbed from the intestine?

2. A definite amount of blood serum is injected intravenously. Observe that albuminuria does not result.

4. A solution of egg albumin, containing approximately the same amount of coagulable protein as the blood serum, is injected intravenously. Observe that albuminuria soon follows. Can egg albumin be assimilated and utilized when injected directly into the blood, or is it eliminated as a foreign body?

**154. Glycolytic Action of Shed Blood:** 1. A definite amount (10 grams) of fresh defibrinated dog's blood is mixed with an equal amount (by weight) of a saturated  $\text{Na}_2\text{SO}_4$  solution and acidulated with acetic acid. This is boiled until the foam is white and distilled water added to make 20 grams. This is then filtered and the filtrate is gradually added from a burette to a definite amount of Fehling's or Purdy's sol. while the latter is boiled in an evaporating dish. Enough of the filtrate is added to cause the blue color to completely disappear. The amount of sugar, as indicated by the quantitative method given above, may be taken as the normal for comparison with the following:

2. Defibrinated blood that has been standing for three or four hours after removal from the same animal, treated in the same way, shows less sugar than fresh blood.

3. Blood from the same animal that has been standing for eighteen or twenty-four hours shows very little or no sugar when treated in the same way. Record the results.



**155. Puncture Diabetes:** A rabbit has been fed upon a liberal allowance of carbohydrates for a day or two before the experiment. The normal urine is collected before the operation and examined for sugar.

The floor of the fourth ventricle (Bernard's so-called diabetic center) is punctured with a blunt probe. After an hour or two the urine is expressed from the bladder and shows pronounced glycosuria.

Write a brief account of this form of glycosuria, telling how it is produced, the conditions necessary for its production, the influence of vasodilatation in other parts, and whether or not hyperglycemia is present.

**156. Phloridzin Diabetes:** The urine of a dog is collected and examined for sugar. Phloridzin is then given (2 or 3 grams) either by the stomach or hypodermatically. Several hours later the urine shows an abundance of sugar when examined by Fehling's solution, Barfoed's reagent, or the phenylhydrazine test.

The sugar content of the blood is also determined. The proteins of blood must be removed before the quantity of sugar can be determined by its reducing power. This may be done as indicated above, or by the following method: Fresh blood is boiled with five times its weight of a mixture of phosphotungstic acid, 70 grams, and HCl (Sp. Gr. 1.20) 20 c. c., in a liter. It is then filtered, neutralized and diluted to a definite volume.

Write a brief account of this form of glycosuria, telling how it is produced, how long it lasts, and whether the amount of sugar in the blood is changed or not. Does the amount of sugar given off in the urine in this form of diabetes depend upon the amount of glycogen stored in the tissues?

**157. Pancreatic Diabetes:** This may be produced by removing seven-eighths or more of the pancreas. All the phenomena of true diabetes (glycosuria, polyuria, emaciation, intense thirst, acidosis, etc.) occur in depancreatized dogs that survive the operation for some time. In pancreatic diabetes there is a condition of hyperglycemia.

**158. Adrenalin Glycosuria:** The hypodermatic injection of



adrenalin chloride (1-3 c. c. of 1-1000 sol. diluted with physiological salt sol.) causes a transitory glycosuria which only lasts a few hours.

### 159. The Nervous Mechanism of Salivary (Submaxillary)

**Secretion:** The submaxillary gland of a large dog is exposed and a cannula is tied in its duct; the chorda tympani nerve and the sympathetic fibers about the artery are also exposed so that they may be stimulated. Count the number of drops for two normal periods of one or two minutes each.

1. *Reflex Stimulation:* Some diluted acetic acid is applied to the mucous membrane of the mouth. Observe the increased flow of saliva, counting the number of drops for two periods and recording the result in a protocol of the experiment.

Later in the experiment the chorda tympani will be cut. Note that acetic acid, applied to the mouth as before, fails to excite any flow from the submaxillary gland after section of the chorda tympani, because the "reflex arc" is broken. Record the result of this observation.

2. *The Chorda Tympani Nerve:* (a) Observe the effect of stimulating the chorda (1) on the flow of saliva, and (2) on the vascularity of the gland. Record the results of stimulation in the protocol.

(b) What change in the character of the secretion as determined by the viscosity?

(c) What effect does cutting the chorda tympani have upon the flow of saliva?

(d) What results from stimulating the peripheral end of the nerve? Record the result.

(e) What kinds of nerve fibers go to the submaxillary gland in the chorda tympani? Are these afferent or efferent?

3. *Sympathetic Fibers to the Submaxillary Gland:* What is the effect of stimulating these fibers upon (a) the amount and (b) the character of the secretion? What change takes place in the state of the blood-vessels from stimulation of the sympathetic? What are the functions of the sympathetic fibers to the submaxillary gland?

4. *Effects of Pilocarpine and Atropine:* (a) Observe and record the effect of 0.001—.002 gram. pilocarpine given intraven-





ously, upon the secretions of the submaxillary gland. Count the number of drops for two periods of two minutes each.

(b) Observe the effect of atropine given intravenously in the same dose and record the result.

(c) Can the secretion be increased by stimulating the peripheral end of the chorda tympani after giving atropine? Is the vascularity increased? Would you conclude from this, that the vasodilatation alone was responsible for the increased flow of saliva when the chorda was stimulated before the administration of atropine?

(d) Stimulate the sympathetic nerve trunk after the administration of atropine and record the result. Are the sympathetic fibers paralyzed by atropine as completely as the secretory fibers of the chorda tympani?

(e) Does a second dose of pilocarpine have any effect after the secretion has been stopped by atropine? What is the action of pilocarpine and atropine, respectively, upon the secretory activity of the salivary glands?

**160. Histological Changes in Secreting Glands:** These changes are best seen in fresh, teased specimens, as fixation agents cause changes in the appearance of the cytoplasm and granules within the gland cells. A rabbit is starved for twenty-four hours so that there is an abundance of zymogen granules in the gland cells. This gives a true picture of the gland in the resting stage.

1. The animal is lightly anesthetized and a small piece of the parotid gland cut off. This is teased on slides in the rabbit's saliva. Examine the specimen with the low and also with the high power of the microscope. Describe the appearance of the resting gland and draw a picture of it. Note that the lobules of the gland are distended, the cells being filled with coarse protoplasmic granules which obscure the nuclei and make the outlines of the cells indistinct.

2. Secretion is produced by injecting small doses of pilocarpine from time to time, so as to cause a copious flow of saliva. Small bits of the parotid are clipped off and teased in saliva as before. Examine and describe the appearance of pieces of the gland taken after one hour, one and one-half hours and two hours of very active secretion. Draw pictures of the later stages to



show the appearance of the "discharged" gland. Note that the zymogen granules are much less abundant and are most numerous toward the middle of the lobule; that the nuclei and outlines of the cells are more distinct.

**161. Histology of the Thyroids and Parathyroids:** Review the normal histology of these glands. Observe the closed acini of the thyroid, lined by low cubical epithelium and filled with colloid which stains well with eosin.

The epithelial cells of the parathyroids have relatively less protoplasm and the general arrangement is totally different. The cells are arranged in anastomosing columns in some places; in other parts there is no definite arrangement, the cells being closely packed together with an irregular distribution of vascular connective tissue. Note that there are no distinct acini and that there is no colloid.

Study the sections of an hypertrophied external parathyroid, which remained for several weeks in a dog after the removal of the thyroids (with the internal parathyroids). Observe that distinct acini have developed throughout the gland and the epithelial character of the cells is more apparent. While a colorless, homogenous substance is present in the lumen of some of the acini, there is no evidence that this is colloid. The appearance is that of hypertrophy, rather than that of a transformation of parathyroid to thyroid structure.

**162. Thyroidectomy:** Both thyroids, with the internal parathyroids, have been removed from a dog, leaving the external parathyroids intact with their circulation undisturbed.

Observe the condition of this animal from time to time for several weeks after the operation and write a brief account of the results of the experiment. Note that thyroidectomy (without parathyroidectomy) does not produce any symptoms in dogs.

**163. Parathyroidectomy:** All four parathyroids are removed from a dog with the least possible injury to the thyroids. The circulation to the thyroids is not disturbed.

Observe that the animal becomes seriously ill in a very short time. Anorexia is present from the first; tetany begins in a few days, showing itself as fibrillary contractions of the muscles,



especially seen in the tongue; as the tetany grows worse there are muscular twitchings and local spasms; there may be rigidity of the muscles and ultimately general convulsions. At times polypnea is present. Death invariably ensues in a variable time (usually two days to two weeks).

These symptoms are the same as those which follow "total thyroidectomy" in dogs, i. e., complete excision of both thyroids and all four parathyroids. Formerly these symptoms were attributed to the removal of the thyroids. From the experiments that you have seen, which set of bodies would you consider the more essential to life? What causes the tetany and death when both sets of bodies are removed?

Write a brief account of the results of the parathyroidectomy and compare it with a third dog in which both sets of bodies are removed.

**164. Influence of Transfusion on Parathyroid Tetany:** The transfusion of normal blood to a dog with parathyroid tetany causes a transitory disappearance of the nervous symptoms, but the blood of the dog with tetany does not have any effect when transfused into a normal dog.

**165. Influence of Calcium on Parathyroid Tetany:** Observe the effect of giving full doses of calcium acetate or calcium lactate to a dog suffering from severe tetany following parathyroidectomy. Does it modify the severity of the nervous symptoms or cause them to disappear? Does it prolong the life of the animal? Write an account of the experiment.

#### EXPERIMENTS ON THE KIDNEY.

**166. Pressure in the Ureter:** The ureters are exposed through lumbar incisions and cannulas are tied in them. One of the ureters is connected to a mercury manometer with a T-tube interposed between the two. Physiological salt solution is injected into the ureter by means of the side arm of the T-tube until the pressure in the manometer begins to rise. Observe and record the ureter pressure which is sufficient to stop the further formation of urine. How does this pressure compare with the capillary blood-pressure?





**167. Saline Diuretics. (1) NaCl Diuresis:** After the flow of urine returns to the normal, a hypertonic solution of NaCl (1.5 per cent solution) is injected intravenously. Observe the increased flow of urine which lasts for a little time. The urine is collected for five-minute periods as before. Record the results. Observe the change in the character of the urine. This may be determined by taking the specific gravity and also by determining the amount of urea given off for each period. Record the results and compare them with the normal urine collected at the beginning of the experiment.

Observe that the urine formed during NaCl diuresis contains dextrose. It has been found by experiment that there is no hyperglycemia as the result of injecting NaCl solution. How do you explain the *NaCl glycosuria*?

Sodium sulphate when injected intravenously causes a greater diuresis than sodium chloride.

**168. (2) Dextrose Diuresis:** After the flow of urine returns to the normal, a concentrated solution of dextrose (10 to 20 per cent solution) is injected. Observe the tremendous increase in the flow of urine. Record the results for five minute periods. Note that the specific gravity is lowered and the output of urea for the five-minute periods is diminished as before.

It has been found that a preliminary bleeding to the extent of the amount of water attracted into the blood by the osmotic pressure of the dextrose injected, prevents the diuresis to a large extent.

From this it appears that crystalloid diuretics act as such, to a large extent, by producing hydremic plethora and the consequent increase in the capillary blood-pressure. The NaCl glycosuria indicates that the permeability of the renal epithelium is also changed.

**169. Influence of Changes in the Circulation Upon the Formation of Urine:** The normal urine is collected for two or three periods of five minutes each. Make a protocol of the experiment, recording the quantity of urine, the specific gravity and the urea content for each of these periods. The blood-pressure is taken in the carotid artery.



1. Adrenalin is then injected intravenously in small quantities to keep the blood-pressure elevated above the normal. Observe that the excretion of urine is decreased, in spite of the increased blood-pressure, because the renal arterioles are constricted. Record the result.

2. After the flow of urine returns to the normal, alcohol, properly diluted, may be injected. The flow of urine is increased, although the general blood-pressure is not increased, and may even be lowered. The increased formation of urine may be explained by the vasodilatation and the increased velocity of the blood stream (acceleration of the heart). Record the results in the protocol.

**170. Influence of the Splanchnic Nerve on the Renal Vessels and Flow of Urine:** The number of drops of urine falling from the cannula in each ureter is counted for several periods of one minute each. Record the results.

The left splanchnic nerve is then cut just above the diaphragm. Count the drops from each ureter as before and record the result. Note the increase in the flow from the left kidney and explain the cause of it.

The peripheral end of the cut splanchnic is then stimulated. During the stimulation count the drops from each ureter as before and record the result. How do local vasoconstriction and local vasodilatation influence the flow of urine? Make a complete protocol of the experiment.

**171. Influence of Lowered Blood-pressure Upon the Excretion of Urine:** Record the amount of urine excreted from both kidneys in a five-minute period. A transverse section of the spinal cord is made in the upper cervical region to lower the arterial blood-pressure. Artificial respiration is maintained. Observe whether or not any urine is formed after the mean arterial pressure (measured in the carotid) falls below 40 mm. Hg. Record the result and compare with the formation of urine before cutting the spinal cord. What arterial pressure is necessary for the formation of urine?

**172. Specific Diuretics:** The normal urine of another dog is collected for several periods of five minutes each; the specific



gravity is taken and the output of urea for each period is determined.

Caffeine or diuretin (theobromine salicylate) is injected intravenously. The urine is again collected for three periods of five minutes each; the specific gravity and urea output are determined as before. Record the results in a protocol of the experiment. Observe (a) that the quantity of urine is increased; (b) the specific gravity is lowered; (c) the urea output is increased. How does this compare with the urine excreted during saline diuresis?

The diuretic action of caffeine or diuretin is much more pronounced on rabbits than on dogs.

Do the specific diuretics seem to act upon the kidney by changing the physical forces concerned in the formation of urine, or by some specific action upon the renal epithelium?

**173. Urea as a Diuretic:** A solution of urea is injected intravenously after the flow of urine returns to the normal. Observe that the flow of urine is greatly increased; the specific gravity is lowered. Record the results. Observe that urea does not show any toxic effects, even when injected in large quantity. It seems to act as a crystalloid diuretic by producing hydremic plethora. Possibly the permeability of the renal epithelium is also altered as glycosuria often follows the injection of urea as it does after the injection of NaCl.

**174. Compression of the Blood-vessels of the Kidneys:** The kidney is exposed on one side and the renal vein is separated from the other vessels. The renal vein is compressed between the fingers for one minute. Observe that the flow of urine stops.

The other kidney is exposed in the same way and the renal artery is compressed between the fingers for one minute. Observe that the flow of urine stops for a considerable period.

## MUSCLE AND NERVE.

**175. The Nerve-Muscle Preparation:** Pith a frog and destroy the brain and cord. Wrap a towel around the hind legs and hold them in the left hand, the ventral surface being down.





With stout scissors transfix the body at about the fifth or sixth vertebra and divide the spinal column. The viscera, together with the anterior and ventral parts of the body, fall away from the pelvis and the dorsal part. Now remove the viscera by cutting the abdominal walls with scissors, parallel to the vertebral column and close to the pelvis. Strip the skin down over the lower end of the trunk; with scissors cut through the skin about the anus; now continue to pull the skin until the posterior extremities are completely denuded.

Holding the thigh muscles between the thumb and the fingers, carefully tear the fascia between the biceps and semi-membranosus muscle with a seeker and separate the deeper muscles until the sciatic nerve and the femoral vessels come into view. Clear the nerve with a seeker, or a glass rod drawn to a smooth point, taking care not to stretch or injure it in any way. (The nerve should not be handled with forceps.)

Divide the piriformis and ilio-coecygeus muscles and free the nerve up to the last vertebra by lifting the tip of the urostyle (coecyx) and cutting it away. A branch of the sciatic nerve coming off at the upper part of the thigh must be divided with the scissors. Now cut the sciatic nerve as high up as possible. Observe that the muscles supplied by the nerve contract when the nerve is cut.

Separate the tendo-Achillis and sever it below the fibro-cartilage. Lift the tendon and free the gastrocnemius as far up as the lower end of the femur. With stout scissors cut across the knee joint, removing the tibio-fibula with its muscles and the foot. Now lay the nerve on the gastrocnemius and cutting through the muscles of the thigh, divide the femur about its middle. The nerve and muscle should be kept moist with physiological salt solution and the nerve should never be allowed to touch the skin. If the preparation is not to be used at once it should be wrapped in filter paper moistened with physiological salt solution and must not be allowed to dry.

Arrange the preparation in the moist chamber by fastening the end of the femur in the clamp; lay the nerve on the platinum electrodes, handling it with a camel hair brush moistened with physiological salt solution. The tendo-Achillis should be





attached to the muscle lever by means of an S-shaped hook passed through the tendon above the sesamoid bone. Attach a 20-gram weight to the wire which lies in the groove of the axis-wheel of the lever, by means of a wire around the neck of the weight, and adjust the screw beneath the muscle lever so that it supports the weight until the muscle is taut but not stretched. A muscle that is thus loaded only during contraction and not kept on a stretch while at rest, is said to be "after loaded."

**176. Different Forms of Stimulation:** (a) *Mechanical Stimulation* may be applied by pinching the free end of the nerve with forceps. Observe that the contraction results just as it does after cutting the nerve or its branches.

(b) *Chemical Stimulation* may be produced by dipping the end of the nerve in glycerine or a concentrated solution of sodium chloride. A chemical stimulus should only be applied in a watch crystal to the free end of the nerve and the part of the nerve affected should be cut off with the scissors.

(c) *Electrical Stimulation* may be applied in the form of make and break induction shocks or by the make and break of the constant (galvanic) current.

(1) *Single Induction Shocks. Minimal, Submaximal and Maximal Contractions:* Arrange the inductorium for single induction shocks with a simple key in the primary circuit; connect the binding posts of the secondary coil with those of the electrodes in the moist chamber.

Start with a very weak current which can not be felt; gradually change the position of the secondary coil until the induction shocks at the break of the current in the primary begin to be felt. Note the position of the secondary coil and record the height of the contractions on the drum while it is standing still.

Move the drum a short distance by hand after each contraction and take a series of tracings to show the increase in the height of the contractions as the strength of the stimulus is gradually increased. Mark the contractions that occur at the make (m) and at the break (b) of the current in the primary. Increase the strength of the stimulus until the make and the break contractions are of the same height. Observe (1) that the induction shock at the break of the current in the primary coil is a



much stronger stimulus than that at the make; (2) that the height of the contraction increases with the strength of the stimulus up to a certain point (maximal contractions), but beyond that further increase in the strength of the stimulus does not influence muscular contraction. When the electro-magnet is shunted off from the primary circuit the make of the current in the primary may give a stronger induction shock than the break.

With the secondary coil in a position to cause maximal contractions, close the short-circuiting key in the secondary, and make and break the primary current. Observe that the shocks, which are induced momentarily when the current is made or broken in the primary, are not felt by the nerve when the short-circuiting key is closed in the secondary.

Open the short-circuiting key and place the secondary coil just beyond the position at which the minimal contractions were obtained with the weak break shocks. Make and break the primary circuit at intervals of a second or less. Observe that a summation of subminimal stimuli may cause contraction although single stimuli are inadequate.

(2) *Galvanic or Constant Current:* Connect the wires from the battery directly to the binding posts of the moist chamber with a simple key interposed for making and breaking the current. Make and break the current. Observe that contraction occurs at make and at break, but not during the flow of the constant current. Any sudden variation in the intensity of the current may act as a stimulus.

If contraction occurs equally well both at make and break, reduce the strength of the current by using a single battery or by increasing the resistance to the flow of current. This may be done by causing the current from one dry cell to pass through the fine wire of the secondary coil. If the current obtained in that way is not weak enough to cause contraction at make and not at break, it may be still further reduced by connecting the wires from the battery to a rheocord with a key interposed and gradually increasing the strength of the current passing from the rheocord to the electrodes by moving the slider of the rheocord so that the resistance is increased. Observe that the make



of a weak or moderately strong galvanic current is stronger than the break.

**177. Polarization of the Electrodes:** Lead off copper electrodes from the short-circuiting key connected with the batteries. Lay the nerve on the electrodes and open the short-circuiting key. Allow the current to pass through the nerve for three to five minutes; then quickly detach the wires from the batteries; open and close the key repeatedly. Does contraction occur at the make and break of the circuit? Stimulation in this case is due to the electrolytic change in the nerve (polarization). Platinum electrodes show much less polarization than those of copper or other metals, but when the constant current is passed through a nerve, non-polarizable electrodes of clay are used.

**178. Extensibility and Elasticity of Muscle:** Fasten the femur of a muscle preparation securely in the large femur clamp and by means of the S-shaped hook fasten the tendon to the lever. Lower the after-loading screw so that the muscle can extend. With the lever a little above the horizontal line bring it to write on the drum and turn the latter by hand for a distance of one-fourth of an inch. Now place a 20-gram weight in the pan under the lever and turn the drum the same distance as before. Remove the weight and again turn the drum as before. Is excised muscle perfectly elastic, i. e., does it return to its original length when the extending force is removed?

Now place successively 20, 40, 60, 80, 100 and 120 grams in the weight pan, turning the drum by hand the same distance after each extension of the muscle. Does each increase of weight cause the same amount of extension of the muscle?

Now remove the weights, taking 20 grams off at a time, and take a tracing after the removal of each weight to show the return of the muscle. Does it return to the normal immediately after all the weights have been removed? Is excised muscle as perfectly elastic as a muscle in the living body?

Repeat the experiment, using a spiral wire spring instead of a muscle, taking tracings as before. State the difference between the extensibility of muscle and that of a spring.

**179. The Simple Muscle Contraction or Twitch:** Arrange





a fresh nerve-muscle preparation in the moist chamber and place the nerve on the platinum electrodes. Arrange the inductorium for single induction shocks with the simple key in the primary and the electro-magnet as a shunt to mark the point of stimulation. After-load the muscle lever with a 20-gram weight. Find a strength of current which gives a maximal contraction, both at make and at break, and bring the muscle lever and electro-magnet to write *exactly* in the same vertical line.

Set the drum at its fastest speed and let it make one turn. Cause the tuning fork to vibrate and bring it to write on the drum under the muscle lever. Each complete wave marks one one-hundredth of a second. While the drum is turning at its fastest speed stimulate several times with single induction shocks, taking care that the muscle relaxes after each contraction before the next stimulus is thrown in. What time is occupied by the entire simple muscle twitch and by each component part of it?

Now take tracings in the same way, with the drum at the fastest speed, of contractions caused by the make and break of the constant or galvanic current by detaching the wires to the moist chamber from the secondary coil and connecting them with the binding posts of the primary circuit from which the electro-magnet is shunted. Observe that the time of the simple muscle twitch is the same whether caused by the induced or the constant current.

**180. The Action of Curare and the Independent Irritability of Muscle:** Pith a frog and destroy its brain. Expose the left sciatic nerve for a short distance in the thigh, taking great care that the nerve does not touch the skin and that the blood vessels about the nerves are not injured. Pass a ligature beneath the nerve and tie it tightly about the thigh near the knee so as to cut off the circulation in the leg below. Moisten the nerve and keep it from coming in contact with the skin.

With a pipette inject into the dorsal lymph sac one or two drops of a 1 per cent solution of curare. After a few minutes the frog ceases to draw up the right foot when it is pinched or otherwise stimulated. (If necessary inject more curare, but be careful not to use any more than is necessary to cause motor paralysis.)



Arrange the induction coil for single shocks with the hand electrodes attached to the secondary coil. Expose both sciatic nerves up to the pelvis, taking care that the nerves do not touch the skin. Use a strength of current that can be felt by the tongue. Stimulate the right sciatic nerve and observe that it does not cause contraction if enough curare has been given to cause complete paralysis.

Now remove the skin from both legs and stimulate the gastrocnemius muscle of the right leg directly by applying the hand electrodes. Does contraction result?

Now apply the electrodes to the left sciatic nerve above the level of the ligature which was tied around the left thigh. Observe that this nerve trunk has not been paralyzed, although the curare has reached it through the blood and lymph. If curare does not act upon the nerve or muscle fibers, how does it cause paralysis?

Does the muscle possess irritability independently of the nerve fibers and motor nerve plates?

**181. Comparative Irritability of Muscle and Nerve:** Using the same curarized frog, place the electrodes beneath the left sciatic nerve so as to stimulate the left gastrocnemius indirectly, i. e., through its nerve. Find the minimal stimulus which will cause distinct contraction. Now stimulate the right gastrocnemius with the same strength of stimulus, applying the hand electrodes directly. Does it contract? Increase the strength of the stimulus gradually until contraction occurs. Observe that the irritability of the muscle is considerably less than that of the nerve, even when the latter is reduced below the normal by anemia.

Now apply the electrodes directly to the left gastrocnemius and find the minimal strength of stimulus which will cause contraction. Apply the same strength of stimulus to the right gastrocnemius and observe that it is not felt because the motor nerve plates have been paralyzed. Increase the strength of the stimulus gradually until contraction occurs. Ordinarily when an electrical stimulus is applied directly to a normal muscle, is it the muscle or the nerve plates that are stimulated?



**182. Influence of Veratria Upon Muscle Contraction:** Pith a frog and destroy its brain. Into the dorsal lymph sac inject four or five drops of a 1.0 per cent solution of veratria sulphate. Wait a few minutes until the characteristic effect upon the muscle shows itself by the prolonged contraction and slow relaxation which follows when the toes are pinched.

Now take a nerve-muscle preparation and after-load the muscle as usual. Arrange the induction coil for single induction shocks with the electro-magnet as a shunt in the primary circuit. Adjust the secondary coil to give maximal break contractions. Bring the electro-magnet to write on a vertical line with the muscle lever. Set the drum at the fastest speed and let it make a complete turn; stimulate with a single induction shock and wait until the muscle relaxes completely before stimulating a second time. While the drum is going at the same speed take a time tracing in tenths of a second or in seconds. Observe that the latent period and height of contraction are unchanged, but the period of relaxation is enormously prolonged. Observe that a second slower contraction follows shortly after the summit of the first shortening. Record the time in seconds occupied by the phase of relaxation and compare it with the normal.

**183. Influence of Load Upon Muscular Contraction:** Make a fresh nerve-muscle preparation and place it in the moist chamber. Arrange the inductorium for single induction shocks by contact through the kymograph and a platinum foil. Adjust the secondary coil so that the break shocks cause maximal contractions and the make is not felt. With the drum at fastest speed take a tracing with the lever after-loaded only with the heavy weight pan, which weighs 20 grams.

Now move the drum on the upright tube so that the tracings will fall at another point on the drum. Take another tracing with the weight pan alone. Mark the exact point of stimulation on the tracing by moving the drum slowly by hand so that a vertical line is made by the muscle lever at the point where the current in the primary circuit is broken. With the point of stimulation and the muscle lever in exactly the same position, take tracings successively with 1, 3, 5 and 6 20-gram weights in the weight pan, i. e., with loads of 40, 80, 120, 140 grams, re-



spectively. Neither the point of contact in the primary circuit, nor the position of the tracings should be changed while the contractions are recorded. Let the muscle lever mark the base line beneath the tracings and, with the drum at the same speed as before, take a time tracing in hundredths of a second with the tuning fork under the other tracings.

Before varnishing the tracing, mark on each curve the amount of weight lifted. Observe (1) the effect of increasing the load upon the *height* of the contractions; (2) the change in the *time* occupied by the different parts of the myogram.

#### 184. Calculate the Work Done With Different Loads:

After the tracing has been varnished and dried, measure in millimeters the height of each curve from the base-line. Multiply this by the total load lifted in each case, remembering that the weight pan weighs 20 grams. Divide the product by the magnification of the lever as the actual shortening of the muscle is much less than the height of the tracings. This is determined by dividing the length of the long arm of the lever (i. e., the distance in millimeters from the fulcrum to the writing style) by the length of the short arm (i. e., the distance from the fulcrum to the hook to which the muscle is attached). The work done in gram-millimeters would be determined by the formula:

$$\text{Work} = \frac{\text{Load} \times \text{height of tracing}}{\text{Magnification of the lever.}}$$

Observe that a moderate load improves the contraction, but that an excessive load rapidly diminishes the height of contraction. Note that the greatest amount of work is not done with the greatest degree of shortening of the muscle. Make a protocol of the work done with each load:





| Height. | Load.      | Work. |
|---------|------------|-------|
|         | 20 grams.  |       |
|         | 40 grams.  |       |
|         | 80 grams.  |       |
|         | 120 grams. |       |
|         | 140 grams. |       |

### 185. Influence of Temperature on Muscular Contraction:

Isolate the gastrocnemius without the nerve. Fasten the femur, or the tissues about the knee, to the end of the rod in the lower part of the warming chamber. From the hook in the tendo-Achillis lead a fine copper wire up through the opening in the top of the muscle warmer and fasten it over the pulley on the muscle lever, so as to record the contractions. See that the wire is taut and that the muscle is after-loaded with a 20-gram weight. Fasten the glass tube of the muscle warmer in place.

Arrange the inductorium for single induction shocks with the electro-magnet as a shunt in the primary circuit to mark the point of stimulation. See that the muscle lever and the electro-magnet write on the same vertical line and take a tracing of the normal muscle contraction at the room temperature with the drum at fast speed. Record the temperature under the tracing.

Now insert the thermometer into the muscle warmer so that the bulb is near the muscle, but does not touch it or the glass. Take a mixture of ice and salt in a beaker and surround the glass with it until the temperature of the air inside the chamber has been lowered 6° or 8° C. Take another tracing with the drum at the same speed as before and record the temperature under each tracing. As the temperature continues to fall, take a tracing for each reduction of 6° or 8° C until the temperature is reduced to 0°C.

After the temperature has been lowered almost to the freezing point, gradually warm the glass back to the room temperature by



immersing it in tap water at first; then heat a beaker of water carefully until the temperature of the air in the muscle chamber finally reaches  $40^{\circ}\text{C}$ . Take tracings of the muscle twitch at  $25^{\circ}$ ,  $30^{\circ}$ ,  $35^{\circ}$  and  $40^{\circ}\text{C}$ , with the drum at the same speed as before. Then gradually increase the temperature still farther until heat rigor begins. Observe that the muscle begin to shorten at  $41^{\circ}$  to  $45^{\circ}\text{C}$  and gradually continues to do so until a greater degree of shortening occurs than can be produced by the contraction of muscle. This is heat rigor and the properties of irritability and contractility are lost. The drum should be set at very low speed with low gear as soon as the heat rigor begins, so as to take a tracing of the shortening.

The change in reaction of the muscle from alkaline to acid may be shown with the onset of heat rigor by injecting acid fuchsin into the lymph sac of the frog some time before its muscle is used for this experiment. This indicator is harmless and does not influence muscular contractions. When an acid reaction occurs a distinct rose red color develops.

Briefly describe the changes produced in the muscular contraction by heat and cold, respectively, stating the temperature at which maximal contraction occurred and the temperature at which heat rigor began.

#### **186. The Myogram Taken With the Drum at Fast Speed:**

Arrange for single induction shocks by contact between a piece of platinum foil fastened to a spring on a separate stand, and a platinum wire tip attached to a brass rod which is held in a block beneath the drum. Fasten one wire from the batteries to the binding post on the spring holding the platinum foil: connect the other wire from the batteries, through a simple key, with the base of the kymograph by means of the thumbscrew on one side.

Adjust the brass rod in the block so that the platinum wire tip just touches the platinum foil as the drum revolves. Screw the drum up free from the friction disc by means of the screw at the top of the central post which holds the drum.

Arrange a fresh nerve-muscle preparation in a moist chamber. Find a strength of current which causes maximal break shocks, without contraction at make. Bring the drum to the position



where contact is made between the platinum wire and foil; mark the point of stimulation by closing the key in the primary circuit and causing the muscle lever to write in a vertical line. Arrange the tuning fork to mark hundredths of a second just under the muscle lever.

One student should spin the drum by hand, and as it revolves, he should close the key in the primary circuit until one stimulus is applied. As soon as one contraction occurs, the key should be opened. At the same time, while the drum is revolving at the same speed, the other student should take a tracing of the tuning fork vibrations beneath the muscle contraction. As soon as the drum makes one complete turn, the tuning fork should be taken away so that the tracings do not overlap.

Observe that the single muscle contraction only occupies 0.10 second. After the tracing has been varnished drop vertical lines and measure the time occupied by (a) the latent period, (b) the period of contraction and (c) the relaxation. Record your results.

**187. The Refractory Period in Voluntary Muscle:** Adjust two rods with platinum tips in blocks under the drum so that they will strike the platinum foil within 0.01 of a second when the drum is turned at the same speed as in the last experiment. Raise or lower the drum so that the muscle lever will write on another part. With the key in the primary circuit open, turn the drum around once to make a base line. Find the strength of current which causes maximal break shocks and, turning the drum very slowly by hand, mark the two points of stimulation by vertical lines caused by maximal contractions.

Spin the drum at fast speed as before. After one turn of the drum with the key closed, open the key in the primary and take a tracing of hundredths of a second with the tuning fork while the drum is moving at the same speed as before. Observe that when a second maximal stimulus is applied during the latent period there is no change in the height or duration of the contraction, i. e., the second stimulus is not felt. There may be summation of two submaximal contractions falling within the latent period.

**188. Summation and Superposition of Contractions:** (1) With the apparatus arranged as in the last experiment, separate





the platinum tips of the rods farther apart so that the second stimulus will fall during the period of contraction. Mark the points of stimulation by making contact with each of the wires as before, and take a tracing on another part of the drum while it is spinning at fast speed as in the previous experiments. Note that when the two stimuli fall during the period of contraction there is a summation, i. e., the two stimuli are "summed up" to cause a greater single contraction than can be produced by any single stimulus.

(2) Separate the two points of stimulation still farther apart, so that the second stimulus falls early in the period of relaxation. Mark the two points of stimulation as before, and while the drum is spinning at fast speed, take a tracing on another part of the drum.

Observe that the second contraction begins after the latent period which follows the second stimulus, from whatever point the lever happens to be when the stimulus is felt, i. e., the second contraction is superimposed on the first one.

Observe that the refractory period of voluntary muscle is limited to the latent period and that stimuli falling during the period of contraction are felt. How does this compare with heart muscle? (See experiment 89, page 138.)

**189. Tetanus:** Arrange the inductorium for single induction shocks; place the tetanometer and a simple key in the primary circuit. The tetanometer can be made to vary the rate of stimulation by changing the length of the spring; also by changing the strength of the induction shocks used, so that stimulation occurs both at make and break or only at the break.

Find a strength of stimulus which causes *maximal contractions both at make and break*. Set the clock work of the drum for fastest speed in each of the following tracings and by means of the short circuiting key in the secondary circuit only stimulate for a few seconds at a time so that the muscle does not become fatigued. Take short tracings in turn of the muscular contractions caused by 8, 12, 16, 20, 24, 32, and 40 stimuli per second. (See that the strength of the stimulus used each time the rate of interruption is changed, is sufficient to cause contractions, both at make and break, so that the number of stimuli will be double the num-



bers on the tetanometer.) Record the rate of stimulation under each tracing. Take a time tracing in seconds with the drum at the same speed as before.

Write a description of complete and incomplete tetanus, respectively, stating what rate of stimulation is necessary to produce the former.

A muscle that is fatigued is more easily tetanized than a fresh muscle, because the time occupied by each twitch or contraction is prolonged by fatigue. A fresh muscle-nerve preparation stimulated successively by 16, 24, 32, and 40 stimuli per second shows less complete tetanus than a muscle which has been gradually fatigued.

**190. Fatigue of Muscle; the Change in the Myogram From Fatigue:** Make a fresh nerve-muscle preparation and arrange it in the moist chamber with a 20-gram weight as an "after-load."

Lead one wire from the batteries through a simple key to one of the posts of the induction coil for single induction shocks. Fasten the other wire from the batteries to the binding post connected with the platinum foil. Connect the base of the kymograph with the other binding post of the primary circuit for single induction shocks. Adjust the platinum foil so that each arm of the contact-maker at the top of the drum touches it in passing without catching.

Find a strength of current which will cause maximal contractions at the break, while the make shocks are not felt. When the drum is revolving at the fastest speed, the contact-maker will produce a stimulus every  $1\frac{1}{4}$  seconds. See that the clock work of the kymograph is wound up, and with the key in the primary open, let the drum revolve once to make a base-line. Now close the key in the primary and, turning the drum by hand, mark the point of stimulation by vertical tracings at each point of contact.

Set the drum going at its fastest speed and let it make one turn before closing the key in the primary circuit. Without changing the position of any of the apparatus, close the key and let it continue until the muscle is completely fatigued and fails to contract when stimulated. Watch the muscle lever during the



experiment, and if necessary, increase the strength of the stimuli slightly by shoving up the secondary coil, or adjust the platinum foil, so that a contraction results from each contact. After the muscle is completely fatigued take a time tracing in tenths of a second with the drum at fastest speed.

Observe the changes in the character of the contractions as fatigue develops: (a) the diminution in height; (b) the disproportionate prolongation of the relaxation period; (c) the sluggish contraction, and (d) the prolonged latent period.

**191. The Effect of Load on Fatigue:** (1) Make a fresh nerve-muscle preparation and after-load with a 20-gram weight. Disconnect the wires from the base of the kymograph and platinum foil and lead wires from the time-marking binding posts on the table through two dry cells and a simple key to the binding posts of the inductorium for single induction shocks. These will be made at intervals of one second by the large clock.

Set the drum for very slow speed by loosening the thumb screw at the base of the kymograph and shifting the brass rod to change the gear. Now set the drum at such speed that the contractions make separate and distinct vertical lines with very little space between them. Only the height of the contractions and the time necessary to produce complete fatigue are recorded in this experiment.

Find a strength of current which gives maximal contractions at break but none at make. See that the clock work of the kymograph is wound up and let the drum revolve slowly until complete fatigue results from the frequent stimuli, i. e., until the muscle no longer shortens on stimulation.

If the muscle is allowed to rest for a few minutes before starting the tracing, the first few contractions increase in height, like a staircase. This is due to the improvement from exercise.

Somewhere in the series of contraction the condition of contracture or maintained contraction which is so characteristic of fatigue may be seen by the incomplete relaxation and failure of the lever to return to the base line. This may occur early or late in the development of fatigue, the muscles of different individuals varying greatly.

(2) Make a fresh nerve-muscle preparation from the other





leg of the same frog whose muscle was used in the last experiment; after-load with 60 grams, instead of 20 grams. Find a strength of stimulus which causes maximal contractions at break but none at make. Set the drum at the same speed as in the last experiment and take a tracing of the contractions produced at the same intervals of time. How does an increase in the load influence the time necessary to produce complete fatigue?

**192. The Seat of Fatigue:** Make a fresh nerve-muscle preparation. Arrange the induction coil for a tetanizing current with a simple key in the primary circuit. Connect the binding posts of the secondary coil with the central binding posts of the pole-changer with its cross wires removed. From one side of the pole-changer lead wires to the electrodes in the moist chamber for stimulating the nerve; from the other side lead one wire to the metal holding the muscle in the moist chamber, and the other to the muscle lever for sending an induced current through the muscle directly.

Find a strength of current which causes maximal contractions when applied to the nerve and also when applied directly to the muscle. Stimulate through the nerve until the muscle ceases to respond. Then reverse the pole-changer and stimulate the muscle directly. Observe that the contraction occurs, showing that the muscle is not as easily fatigued as the nervous structures which control it. Experimentally it has been shown that the motor nerve plates are the first to show fatigue in a nerve-muscle preparation, while the nerve fibers resist the fatigue much longer than the muscle. See experiment 204, p. 300, on fatigue of nerve.

**193. Fatigue in the Living Human Body From Voluntary Contractions:** Arrange the ergograph so that the lever writes upon the drum. Set the drum at very slow speed by changing the gear. Place the hand on the block and with the tapes tie all the fingers securely except the index finger. Fasten the point of the upright rod in the middle hole of the spring and see that the finger-rest is screwed down against the index finger when it is at rest.

Maximal voluntary contractions of the abductor digiti should be made every second until fatigue is complete. The time can





be indicated by beats of the metronome or by the magnet connected with the time-marking current. When fatigue is complete, the belly of the abductor indicis should be stimulated directly by means of single induction shocks passed through it from brass electrodes covered with cotton wet with salt solution, which should be placed on opposite sides of the muscle. Observe that contraction occurs from direct stimulation after voluntary effort fails. Fatigue in the living body first shows itself in the spinal neurones.

**194. Nature of Voluntary Muscular Contraction:** Change the position of the drum so that the ergograph writes upon a clear space and set the latter for fastest speed. Make several maximal voluntary contractions of the abductor indicis. Some of these should be made in the shortest time possible; others should be prolonged for several seconds. Connect the electromagnet with the time current, with the drum at the same speed as before and record seconds under the tracing. Observe (1) the time occupied by the quickest ordinary voluntary contraction and compare it with the time of the single muscle twitch produced by an induction shock; (2) that the tracings of the prolonged contractions show small waves like an incomplete tetanus, showing that voluntary contractions are made up of separate contractions fused together.

**195. Current of Injury or Demarcation Current:** Destroy the brain and spinal cord of a frog and remove the skin from the hind legs. Dissect out the sciatic nerve without injuring the thigh muscles and cut it as high in the pelvis as possible. (Use a pointed glass rod with a smooth tip instead of a metal seeker and do not let metals touch the nerve or muscle except in cutting them.)

Before cutting the thigh muscles, lift the free end of the sciatic nerve and let it fall longitudinally upon the uninjured surface of the triceps muscle. Observe that no contraction of the gastrocnemius follows, because there is no current in the thigh muscles to stimulate the nerve.

Now cut the thigh muscles transversely a little above the middle of the thigh. Lift the nerve with the glass rod so that its



trunk rests upon the uninjured surface of the muscle and the free end of the nerve falls upon the surface of the transverse section through the muscles. Observe that the contraction of the leg muscles occurs each time the nerve touches these two points of the injured muscle, i. e., the make or closing of the demarcation current stimulates the nerve. Sometimes, when the nerve is very irritable and there is a strong current of injury, contraction also occurs when the current is broken by lifting it from the injured surface. Usually the result is like the stimulation by a weak constant or galvanic current from any other source, i. e., contraction occurs at make but not at break. Repeat the observation a number of times and record the results.

**196. Current of Action:** Make two nerve-muscle preparations leaving the foot and leg attached to each. Only handle the nerves with the glass rod or camel hair brush. Place them on a glass plate. Let the nerve of one (B) rest upon the gastrocnemius of the other (A). Find the minimal induction shocks which produce maximal contractions when the hand electrodes are applied to the nerve of A. Be careful not to use a greater strength of current than necessary in order that there may be no escape of current from the electrodes. Observe that the muscle B contracts each time the nerve of A is stimulated. The nerve of the preparation B is stimulated by the current of action in the muscle of preparation A.

Now use the tetanizing current for stimulating the nerve of A. Observe the secondary tetanus in the muscle B produced by the series of currents of action in the muscle A.

Pinch the free end of the nerve A with forceps. Observe that each contraction of the muscle A produced by the mechanical stimulation of its nerve, causes a secondary contraction in B from the current of action in A.

To prove that the nerve of B does not receive electrical stimulation from the electrodes connected with the induction coil, tie a ligature, moistened with physiological salt solution, about the nerve of A near to the muscle and stimulate the nerve of A with a tetanizing current as before. The ligature crushes the nerve fibers and blocks the transmission of nerve impulses, but does not prevent the conduction of an electrical current, i. e., it



severs the continuity of the nerve physiologically, but not physically.

**197. The Capillary Electrometer:** The current of injury in the voluntary muscle of the frog and the current of action in the frog's heart will also be demonstrated by the capillary electrometer which is used to detect feeble electrical currents. In the one case one non-polarizable electrode is placed on the injured surface and the other on the uninjured surface; in the other case one electrode is placed on the base and the other on the apex of the heart.

One electrode is then connected with the mercury and the other with the dilute sulphuric acid of the electrometer with a short-circuiting key interposed. Note the change in the level of the meniscus of mercury in the capillary tube, as observed under the low power of the microscope, when the short-circuiting key is opened. It always changes from a higher to a lower potential, the direction being the reverse of what it appears to be under the microscope. As soon as the key is closed the meniscus returns to the normal.

## PHYSIOLOGY OF NERVE.

**198. Relative Excitability of Nerves to Flexors and Extensors:** Pith a frog and with stout scissors cut across the spinal column about its middle. Cut the abdominal walls parallel to the pelvis and remove the viscera. Strip the skin from the legs. Lay the preparation on its dorsal surface and place one sciatic plexus on the shielded electrodes.

Arrange the inductorium for a tetanizing current. Starting with the secondary coil at right angles to the primary, gradually increase the strength of the current until feeble contractions occur. Observe that the flexor muscles are the first to respond to the weak stimuli.

Now increase the strength of the current considerably and observe that the extensors are more irritable to strong stimuli than the flexors.

**199. Distribution of the Nerve Fibers of the Sciatic Plexus:** Separate the three roots of the sciatic plexus so that each





one in turn can be stimulated separately, beginning with the upper (outer) root. Use the shielded electrodes and start with induction shocks which are too weak to be felt. Gradually increase the strength of the current until contractions occur. Observe that the upper (outer) root supplies the muscles moving the thigh at the hip joint; the middle root causes motion of the leg upon the thigh at the knee joint; the lower (inner) root controls the lower muscles, causing movements at the ankle joint.

**200. Effect of CO<sub>2</sub> Upon Nerve, Showing That Irritability and Conductivity are Separate Properties:** Arrange the inductorium for a tetanizing current and connect the secondary coil with the central binding posts of the pole-changer without cross wires. From one side of the pole-changer lead off hand electrodes; from the other side lead wires to the needle electrodes in the cork of the gas chamber.

Connect the longer glass tube of the gas chamber with the short glass tube of the wash bottle connected with the gas generator by means of small rubber tubing. Place the gas chamber on a filter paper moistened with physiological salt solution on the cork plate and hold it in position by means of pins pressed into the cork plate.

Now dissect out the sciatic nerve up to the vertebral column in a good-sized frog and cut it as high in the pelvis as possible. Free the nerve down to the knee; then cut across the thigh muscles and divide the femur at its middle, leaving the gastrocnemius intact with the foot and leg. Tie a silk thread to the free end of the nerve, and by means of this, pass the nerve through the holes in the gas chamber, until the knee is close to the chamber. See that the nerve touches the electrodes within the gas chamber. Close the holes in the gas chamber by kaolin moistened with physiological salt solution without injuring the nerve. Fasten the femur in the femur clamp on the frog plate so that the foot is flexed on the leg by its own weight.

Find a strength of current that is just sufficient to cause tetanus (a) when the hand electrodes are applied to the nerve above the chamber, and (b) when the nerve is stimulated by the electrodes within the chamber.



Now pour some 10 per cent HCl into the CO<sub>2</sub> generator through the thistle tube. After the CO<sub>2</sub> has passed through the gas chamber for several minutes, stimulate at both points as before, using the same strength of current. Observe that the stimulation by the electrodes within the chamber fails to cause contraction, or only does so with a very strong current, while practically the same strength of current that was first used causes tetanus when applied to the nerve above the gas chamber. This shows that CO<sub>2</sub> lessens or destroys the irritability of the nerve fibers without changing the conductivity.

Disconnect the CO<sub>2</sub> generator from the gas chamber and stimulate the nerve with the electrodes in the gas chamber again. Observe that the irritability soon returns to the normal after the CO<sub>2</sub> escapes.

Connect an Erlenmeyer flask containing a small amount of alcohol with the gas chamber. Vaporize the alcohol by placing the flask in a pan of hot water. After several minutes stimulate at both points as before, increasing the strength of current if necessary. Alcohol diminishes the irritability somewhat, but almost entirely destroys the conductivity.

Disconnect the rubber tubing from the gas chamber so that the vapor of the alcohol may escape. Again stimulate the nerve at both points as before, and observe that the stimulation above the chamber is again felt, so that the result obtained with alcohol is due to diminished conductivity within the chamber and not to diminished irritability near the free end of the nerve.

## **201. Electrotonus; Effect of the Constant or Galvanic Current Upon the Vital Properties of Nerve:**

### **A. ALTERATIONS IN EXCITABILITY.**

From the binding posts of the time-marking current, lead wires through two dry cells to the binding posts of the primary coil of the inductorium for single induction shocks with a simple key interposed and the electro-magnet arranged as a shunt. Connect the secondary coil with the platinum electrodes in the moist chamber. These electrodes are to be used as the stim-



ulating electrodes to determine changes in the excitability, and should be placed next to the muscle.

From two other dry cells lead wires through a simple key to the central binding posts of Pohl's commutator or pole-changer with the cross wires in. From the two binding posts on one side of the pole-chamber lead wires to the non-polarizable electrodes in the moist chamber. These are the electrodes for producing the changes of electrotonus during the passage of the constant or galvanic current, and must be applied to that part of the nerve farthest away from the muscle.

See that the set screws in the blocks for holding the boot electrodes are placed toward the foot of the boot and not away from it. The boots must be tilted at an angle of 45 degrees with the floor of the moist chamber, so that the heel of the boot is the most dependent part and the toe is level with the top of the boot. This prevents the  $\text{ZnSO}_4$  solution from reaching the toe of the boot. The cup on the foot of the boot must be filled with physiological salt solution and the nerve should rest on the very tips of the toes of the boot electrodes.

Determine the position of the reverser which gives an ascending current, i. e., with the anode nearest the muscle, and mark that side of the commutator "a" with chalk; mark the other side "d" for a descending current, i. e., when the cathode is nearest the muscle.

Make a nerve-muscle preparation with as long a stretch of nerve as possible and place it in the moist chamber with the nerve resting on both pairs of electrodes, the stimulating electrodes from the induction coil being nearest the muscle. Place some moist filter paper in the moist chamber to prevent drying of the nerve.

Bring the muscle lever to write upon the drum with the electro-magnet on the same vertical line beneath it.

1. *Cathelectrotonus or Increased Excitability at the Cathode:*  
(a) Close the key in the primary circuit of the induction coil and find the position of the secondary coil at which minimal break contractions occur as the current is interrupted every second, or every other second, by the clock. Set the drum going at slow speed and take a tracing of several minimal or submaximal contractions until they are uniform in height.





Arrange the reverser of the pole-changer for a descending current. Now close the key of the galvanic, or as it is here called, the polarizing current, for ten to twenty seconds. Observe that the contractions caused by the induced or stimulating currents are greatly augmented and may be maximal, as a condition of cathelectrotonus is produced in the nerve near the stimulating electrodes.

(b) Now lessen the strength of the induced currents by changing the position of the secondary coil until contractions just fail to occur (subminimal stimuli). Set the drum at the same speed as before, with the electro-magnet marking the points of stimulation. Again close the descending galvanic or polarizing current. Observe that the subminimal stimuli now produce contractions, because the irritability is increased in the neighborhood of the cathode, i. e., the nerve near the stimulating electrodes is in a state of cathelectrotonus.

Note.—The galvanic or polarizing current should not be too strong, nor should it be passed through the nerve too long at a time, or the irritability may be lessened after a brief period during which it is increased. Even with a current of moderate strength the increased irritability at the cathode is of short duration and is often quickly followed by the opposite condition. As an after-effect the irritability is decreased after the passage of the polarizing current.

The alteration in the excitability at the cathode is much less constant than that which takes place about the anode.

## 2. *Anelectrotonus or Decreased Excitability at the Anode:*

(a) Increase the strength of the stimulating induction shocks by changing the position of the secondary coil, until the weakest stimulus is found which will cause maximal contractions. Set the drum going at slow speed as before, and take a tracing to see that the contractions are uniform in height.

Reverse the pole-changer, so that the anode is next to the stimulating electrodes, and close the key, causing an ascending galvanic or polarizing current to pass through the nerve. Observe that the induced or stimulating currents, if not too strong, either fail to cause contractions or only produce submaximal contractions as the irritability is lessened in the neighborhood of the anode.

(b) Find the position of the secondary coil which will give stimulating currents of such strength as to cause minimal or





submaximal contractions. Set the drum going at slow speed as before and take a short tracing to get contractions of uniform height. Without stopping the drum, close the key in the ascending galvanic or polarizing current. Observe that the contractions cease. The points of stimulation are indicated by the electro-magnet. Open the key in the polarizing current while the drum is still going, and observe that the condition of anelectrotonus, or decreased excitability, is followed by a condition of increased irritability as an after-effect after the passage of the galvanic current.

#### B. CHANGES IN CONDUCTIVITY.

1. With all the connections from the batteries remaining the same as in the last experiment, place the platinum or stimulating electrodes from the induction coil near the free or central end of the nerve; bring the two non-polarizable electrodes connected with the galvanic current as near the muscle as possible, i. e., between the stimulating electrodes and the muscle.

Find the position of the secondary coil which will give induction shocks of such strength as to cause submaximal or maximal contractions as the current is interrupted in the primary circuit by the time marker. Set the drum at slow speed and take a short tracing.

(a) Without stopping the drum, close the key in the ascending galvanic or polarizing current. Observe that the contractions cease.

(b) Now open the galvanic current and change the reverser of the pole-changer so as to give a descending current. Again close the key in the galvanic current and observe that the contractions cease.

The conductivity of the nerve is lowered by the galvanic current between the muscle and the stimulating electrodes, thus blocking the nerve impulses from the latter. As the conductivity is lowered so as to produce a complete block when the galvanic current is passed in either direction, the failure to get contractions can not be due to the changes in irritability.

2. Now place the stimulating electrodes midway between



the non-polarizable electrodes and place these as far apart as the length of the nerve will permit.

Find the position of the secondary coil which will cause submaximal or maximal contractions. Set the drum at slow speed and take a short tracing.

(a) Without stopping the drum, close the key of the descending constant or polarizing current, i. e., produce a condition of cathelectrotonus between the muscle and the stimulating electrodes which are placed at the nodal point. Observe that the contractions cease.

(b) Open the galvanic current and change the reverser of the pole-changer so that the anode will fall between the muscle and the stimulating electrodes. While the drum is still going, close the key of the ascending constant current and observe that the contractions cease.

This shows that the conductivity is decreased, both at the anode and at the cathode, the degree depending upon the strength of the galvanic or polarizing current. The decreased conductivity at the anode may come on more gradually than at the cathode.

#### C. ELECTROTONIC CURRENT. PARADOXICAL CONTRACTION.

Pith a frog and expose its sciatic nerve. Sever the nerve as high up in the pelvis as possible and trace it down to the knee. Observe the two branches into which it divides. Determine which branch goes to the gastrocnemius and divide the other one as near to the knee as possible.

Stimulate the central end of the divided branch by a galvanic current and observe that the muscle supplied by the other division of the nerve contracts. This is due to the electrotonic current set up in the nerve by the galvanic or polarizing current. Contractions can not be produced by stimulating the divided branch of the nerve by a mechanical stimulus or by induction shocks. The stimulation of efferent fibers can not, therefore, be due to the current of action. This electrotonic current is a physical change, which may be detected by the galvanometer in a dead nerve, while the alterations of the irritability and conductivity produced by the galvanic current are changes of the physiological or vital properties of the nerve.



**202. Pflüger's Contraction Laws or the Stimulating Effect of the Galvanic Current:** Make a fresh nerve-muscle preparation and place it in the moist chamber with the nerve on the non-polarizable electrodes. Tilt the foot electrodes as in the preceding experiment so that the toe is level with the top of the foot. The nerve should rest on the tip of the toe of each electrode. See that there is plenty of moist filter paper in the moist chamber to prevent drying.

Connect the wires from one or two batteries with the central binding posts of the pole-changer with a simple key interposed. From the binding posts on one side of the pole-changer lead wires to the rheocord. From the rheocord, as a shunt, lead two wires to the non-polarizable electrodes in the moist chamber. Arrange the pole-changer for a descending current.

1. Make and break the current, gradually increasing the resistance to the current that passes through the rheocord, until contractions occur at make but not at break.

Now reverse the direction of the current and stimulate as before, gradually increasing the strength of the current shunted off to the nerve by increasing the resistance to the current passing through the rheocord, until the contraction again occurs at make but not at break. This is the response obtained by a *weak current* and shows that the increase in excitability caused by the development of cathelectrotonus at the make is greater than that caused by the disappearance of the anelectrotonus at the break of the galvanic current.

2. Now increase the strength of the current shunted off to the nerve, by increasing the resistance offered to the current from the batteries through a greater length of German silver wire in the rheocord, until the contractions occur both at the make and at the break of the ascending and of the descending current. This is the reaction obtained by a current of *medium strength*.

3. The strength of current should be still further increased. If this can not be done by increasing the resistance of the rheocord still further so as to shunt off more of the current from the batteries to the nerve, the battery current may be





connected directly to the non-polarizable electrodes with the pole-changer and a simple key interposed. Observe that a strong descending current gives contractions at make, but not at break, while a *strong ascending* current gives contractions at break, but not at make.

The contractions obtained with the galvanic current vary with the electrotonic changes produced and depend upon the following conditions: (1) the intensity of the current: (2) the direction of the current: (3) the make or break of the current. Tabulate the results obtained below:

| Strength of Current. | Ascending. |        | Descending. |        |
|----------------------|------------|--------|-------------|--------|
|                      | Make.      | Break. | Make.       | Break. |
| Weak.                |            |        |             |        |
| Medium               |            |        |             |        |
| Strong.              |            |        |             |        |

**203. Reactions With the Galvanic Current in the Living Body:** The galvanic current is used practically by placing a large flat indifferent electrode anywhere on the body, and then a small electrode placed over a muscle or nerve is used as a stimulating electrode. The latter is connected, in turn, with the cathode and the anode. Both electrodes must be covered with cotton wet with strong salt solution so as to lessen the resistance offered by the skin. The result obtained depend upon the electrotonic changes produced at the physiological anodes and the physiological cathodes in each case and the density of the current at the stimulating electrode.

This may be demonstrated in man by using from eight to ten or more dry cells arranged in series. The commutator or pole-changer is used as a convenient means of changing the direction of the current. The stimulating electrode is placed over the ulnar nerve between the internal condyle and olecranon. State the order in which the following occur in the normal body: anodal closing, anodal opening, cathodal closing, cathodal opening.



**204. Fatigue of Nerve:** Arrange a nerve-muscle preparation in the moist chamber. Place the non-polarizable electrodes between the stimulating (platinum) electrodes and the muscles. Stretch the nerve across both pairs of electrodes.

Make another nerve-muscle preparation, but do not isolate the gastrocnemius. Expose the sciatic nerve down to the knee and sever it as high as possible; cut the femur at its middle, but leave the foot and leg attached. Hold the femur so that the nerve of the second preparation rests upon the stimulating electrodes with the free end of the other nerve.

With the ends of both nerves on the stimulating electrodes, find the strength of induction currents necessary to produce tetanus in both preparations. Now make the galvanic current so that the impulses produced by the tetanizing current will not be able to reach the muscle of the first preparation on account of the "block" in conductivity caused by the galvanic current. Stimulate both nerves with a tetanizing current until the muscle of the second preparation ceases to contract and the toes do not move; then break the galvanic current. Observe that contraction occurs in the first preparation.

Both nerves were stimulated by the same tetanizing current until the second preparation showed fatigue. The nerve impulses could not reach the muscle of the first preparation on account of the block produced by the diminished conductivity during the passage of the galvanic current. This shows that the nerve fibers are not readily fatigued, in fact they are not fatigued as easily as muscle.

In a previous experiment on muscle (experiment 192) it was shown that the muscle responds to direct stimulation after it fails to respond to indirect stimulation through its nerve. If the first seat of exhaustion is not in the muscle or nerve, it must be in the motor nerve plates.

**205. Reactions of Degeneration:** The electrical reactions of muscles after degeneration of their nerves will be demonstrated in two dogs. The sciatic nerve of one is cut high up and a small segment is excised to prevent rapid regeneration. The sciatic nerve of the other is merely severed and the ends are allowed to fall in close apposition in the wound so that re-



generation can take place without any difficulty. The electrical response of the paralyzed gastrocnemius as compared with the normal side is studied at intervals of two to four weeks after the operation.

Observe and record (1) the relative excitability of the paralyzed and unparalyzed muscle to electrical stimulation; (2) the irritability to the induced (faradic) current as compared with the constant (galvanic) current; (3) the "qualitative" change with the galvanic current, i. e., the anodal closing contraction (An. C. C.) as compared with the cathodal closing (C. C. C.). Write an account of the reaction of degeneration as observed from time to time in each one and explain the difference between them

**206. Structural Changes During Degeneration and Regeneration of Nerve Fibers:** Study carefully the longitudinal sections of the peripheral end of the sciatic nerve after section of the nerve.

(a) Compare the appearance of the peripheral end in the early stage of degeneration (one week after the section) with the central (normal) part of the nerve. Both sections have been stained in exactly the same way by Pal's method.

(b) Observe the appearance of another nerve showing complete degeneration four weeks after section. The electrical reactions of degeneration were complete in this case.

(c) Study the appearance of a sciatic nerve showing regeneration, twelve weeks after section and primary union. Observe that the fibers of the peripheral end do not differ from the normal fibers of the central end. Note the thickening and cicatricial tissue at the point of section; that many delicate axis cylinder processes grow out from the central end of this scar tissue; many of these can be traced into the peripheral end of the nerve; some have become curled and twisted by the scar tissue so that they do not reach the peripheral end of the nerve trunk. The reactions of degeneration had been complete three weeks after section of the nerve in this experiment. At the time the animal was killed (twelve weeks after the operation) the electrical reactions were normal and the motor paralysis had completely disappeared.





Make drawings and describe the appearance of each of these sections.

### REFLEXES.

**207. Preparation and Observation of a Reflex Frog:** Pith a frog with an ordinary pithing-pin, severing the medulla by moving the pin laterally, and destroy the brain by turning the pin forward into the cranial cavity. Be careful not to injure the spinal cord. The animal is in a condition of shock, i. e., there is a temporary depression or suppression of the spinal reflexes and nervous activities as the result of the sudden severe injury to the central nervous system. Physiological shock only lasts a short time and rapidly disappears, but clinically surgical shock is, as a rule, progressive and is characterized by a greater disturbance of the circulation.

Observe the frog carefully in the condition of shock and compare with a normal frog placed under the bell-jar. Note (1) that the eyes are closed; (2) the posture is quite different; (3) the muscles are completely relaxed so that the extremities are not drawn up to the body; (4) the respiratory movements have ceased and there are no reflex movements even when the foot or toe is pinched. Record your observations.

*Observe the Reflex Frog:* After waiting some time for the condition of shock to pass off, note (1) that the animal does not sprawl out as much as at first; (2) that the extremities are drawn up to the trunk; (3) reflex movements may be produced by stimulating the skin or pinching a toe; (4) the animal does not sit as erectly as a normal frog and may not turn over on the ventral surface when placed on its back (compare this with a normal frog); (5) the eyes remain closed and there is an absence of respiratory movements; (6) note that when one leg is extended from the body it is promptly drawn back to its natural position, but some stimulus is necessary to cause movements, i. e., all activities are now reflex in nature. Spontaneous movements do not occur. Reflex actions may be carried on by the spinal cord alone without the brain. Write an account of your own observations on the reflex frog.

**208. Reflex Action:** Pass an S-shaped hook through both





jaws and suspend the reflex frog from a funnel ring. Pinch one toe feebly with the forceps, repeating the stimulation and gradually increasing the strength of the stimulus. Observe that a feeble stimulus causes slight movements of the part stimulated, and an increase in the intensity of the stimulus causes more extensive movements. Moderate stimulation only causes movements of the part stimulated, but a strong stimulus may cause movements of the other parts, the reflex activities extending (by irradiation) first to the opposite side of the cord and then to other segments of the spinal cord. When the stimulus is applied to the trunk near the median line the movements may be bilateral even from moderate stimulation. Describe the results of this experiment.

**209. The Purposive Character of Reflexes; Co-ordinated Reflexes:** Moisten several small pieces of filter paper (not more than one-fourth of an inch square) with commercial acetic acid in a watch crystal. Have at hand a large beaker of water. With forceps pick up one of the pieces of paper, drain off any excess of the acid, and apply the paper to the skin on the inside of one thigh, taking care that it does not touch the opposite thigh. Carefully observe the movements which result and then quickly wash the acid off by raising and lowering the beaker of water without handling the frog.

Write an account of your observation. What part first showed reflex movements? Was it a simple reflex limited to the part stimulated, or was there irradiation to other spinal centers producing complex reflex movements? Were these movements co-ordinated? Was the reflex purposive or not?

After a rest of five minutes, repeat this experiment, but hold the leg on the side to which the acid is applied. Observe the unsuccessful efforts on the side stimulated, and the co-ordinated movements of the opposite side to remove the irritant. Wash the acid off as before and rest again.

Now apply a piece of paper moistened with acetic acid to the skin of the trunk just above the anus. Observe the movements which are bilateral from the first, and which are co-ordinated in the beginning, but soon become inco-ordinate. Wash the acid off and record your observation.



**210. The Effect of Strychnia Upon the Excitability of the Spinal Centers:** With a fine pipette inject into the dorsal lymph sac of a reflex frog one or two drops of a 0.1 per cent solution of strychnia sulphate. Wait a few minutes for the drug to be absorbed. Now apply a light stimulus to some parts of the body (e. g., pinch a toe with the forceps). Observe that this is not followed by a simple reflex, but by complex, inco-ordinate movements of other parts. A feeble stimulus now causes irradiation throughout the spinal cord and may excite extensive convulsive movements or general spasms. This is due to an increase in the irritability of the spinal centers.

Keep the frog quiet and free from any irritation. Observe that the spasms do not come on spontaneously, but from some slight stimulus which ordinarily would not excite reflex activity. Note that the extensor muscles are affected more than the flexors during the convulsive seizures.

**211. The Action of Chloral in Lessening the Excitability of the Spinal Cord:** With a pipette inject five or six drops of a 1 per cent solution of chloral hydrate into the dorsal lymph sac of the frog used in the last experiment and wait for its absorption.

From time to time stimulate the frog and if the exalted excitability of the spinal cord produced by the strychnia does not disappear, inject some more chloral until that result is obtained.

Stimulate the frog in the same way as in the previous experiment and observe that the excitability of the spinal centers is diminished by chloral.

**212. The Reflex Centers Contained in the Spinal Cord:** Introduce the pithing-pin through the opening made between the skull and the first vertebra and pass it down the vertebral canal as far as it will go, destroying the entire spinal cord. After waiting some time for the shock to pass off, apply different stimuli and observe that it is impossible to obtain any reflex action because the "reflex arc" has been broken by destruction of the reflex centers.

Now remove the skin from the legs and expose the sciatic nerve. Stimulate the muscles and nerve by applying the hand



electrodes from the induction coil and observe that both the nerve and the muscles are irritable. The failure to obtain reflex action can not, therefore, be due to any loss of the vital properties of the nerves or muscles.

**213. Reflex Time as Determined by Türk's Method. Variation in Reflex Activity:** For this experiment use a frog that has been prepared two hours before the exercise so that there will be no delay in waiting for the shock to pass off. The cerebral lobes have been removed by section in front of the optic lobes, so that the latter bodies (representing the basal ganglia of the brain) remain intact with the spinal cord.

Have at hand a beaker of 0.2 per cent sulphuric acid, a beaker containing 0.4 per cent sulphuric acid, and a large beaker of water. See that each one is correctly labeled.

Connect the electro-magnet through two dry cells from the binding posts of the time current on the desk. This current will be interrupted 100 times per minute.

Suspend the frog by an S-shaped hook from a support. With a glass rod gently hold one leg aside and immerse the other foot up to the ankle joint in 0.2 per cent sulphuric acid. Count the number of times the time-marking current is made by watching the electro-magnet, between the application of the stimulus and the beginning of the reflex action in withdrawing the foot. Quickly wash the acid off by raising and lowering the beaker of water, taking care that the frog is not handled and does not touch the beaker. Note the reflex time or spinal latent period in hundredths of a minute. After waiting three or four minutes, repeat the experiment and take the mean of the two observations as the average reflex time with a weak stimulus. Record your results.

After another period of rest, repeat the experiment, using 0.4 per cent sulphuric acid and washing the acid off each time. Make two observations and take the average. Observe that an increase in the intensity of the stimulus decreases the reflex time and may cause spinal irradiation. Record your observations.

**214. Stimulation of the Reflex Inhibitory Centers of the Optic Lobes by Quinine:** Inject into the dorsal lymph sac of





the frog used in the last experiment a few drops of a 2 per cent solution of quinia bisulphate and wait several minutes for its absorption.

Determine the reflex time by Türk's method, using the weaker acid and washing the acid off after each observation. Record the result, making two observations.

Note that the reflex time has been prolonged considerably, showing that the reflex activity of the spinal centers has been depressed.

Now destroy the medulla of the frog by introducing the pithing-pin between the skull and atlas and moving it laterally. This severs the connection between the optic lobes and the spinal centers. After waiting for the shock to pass off, again determine the reflex time as before, taking the average of two observations. Compare the reflex time now with the last observation and record the result.

Observe that the reflex time has been lessened by removal of the optic lobes, showing that the decreased reflex activity under the influence of therapeutic doses of quinia is due to stimulation of the reflex inhibitory centers in the optic lobes (Setschenow's center). Overdoses of quinia may increase reflex activity, thereby lessening the reflex time, by directly stimulating the spinal centers.

### **215. Reflex Time as Determined by Electrical Stimulation:**

Use the same frog as in the last experiment. Arrange the inductorium for single induction shocks with the simple key in the primary circuit. From the secondary coil lead off two very delicate wires and hook their free ends in the web of the right foot, taking care that the wires do not touch and thus short-circuit the induced currents. Wait for the frog to become quiet after the handling and mechanical stimulation of the foot.

Start with *very weak single induction shocks* which are not felt, making and breaking the current by the key in the primary, and gradually increase the strength of the induced currents until the stimuli are felt. Observe that the single induction shocks only cause a local twitching or contraction, but fail to cause reflex movements.

Now arrange the inductorium for a *tetanizing current*, using



the same strength of current that caused local twitchings before. Close the key in the primary, and from the electro-magnet in the time-marking current, count the hundredths of a minute that intervene between the application of the stimulus and the beginning of the reflex movements. If necessary, increase the strength of the current until reflex action is produced. After a rest of a few minutes repeat the observation. Record the strength of the current used and the reflex time as indicated by the average of the two observations. Observe that the reflex time, as determined by electrical stimulation, is less than it is when dilute acid is applied to the skin.

**216. Number of Stimuli Required for Reflex Action:** Let the secondary coil remain in the same position as in the last experiment with the wires attached to the frog's foot. Close the short-circuiting key in the secondary and arrange the inductarium for single induction shocks with the tetanometer in the primary circuit. Arrange the tetanometer, in turn, for 8, 16, 20 and 24 stimuli per second and determine the reflex time with each rate of stimulation, allowing the frog to rest several minutes after each observation. The interruptions by the tetanometer may be started with the short-circuiting key of the secondary current closed: the reflex time is then determined by counting the hundredths of a minute, as indicated by the magnet in the time current, from the opening of the short-circuiting key in the secondary until the reflex movement starts. A determinate number of stimuli is necessary to elicit reflex action. Observe that the higher the rate of stimulation, the shorter the reflex time. Record your results.

**217. Inhibition of the Spinal Reflexes by Stimulating the Optic Lobes:** For this experiment a frog is prepared for each table before the exercise as follows: the frog is etherized and the roof of the skull removed so as to expose the cerebral and optic lobes. The former are then removed.

After the frog has recovered from the shock, determine the reflex time by Türk's method, using 0.2 per cent sulphuric acid. After a period of rest, repeat the observation and take the average of the two as reflex time with Setschenow's reflex inhibitory center in the optic lobes intact.



Now apply one or two crystals of NaCl to each optic lobe, taking care to remove any fluid or blood by sponging before the salt is applied so that it does not reach the medulla or spinal cord. Again determine the reflex time in the same way as before, and observe that it is greatly prolonged as the result of the inhibition of spinal reflexes caused by the chemical stimulation of Setshenow's center in the optic lobe. Record your observations.

Now destroy the medulla by pithing between the skull and atlas, thus severing the connection between the optic lobes and the spinal cord. After the shock has passed off, again determine the reflex time, making two observations, and compare with the results obtained before. Record your results.

**218. Inhibition of Reflexes by Simultaneously Stimulating Different Parts:** Suspend a reflex frog from a support and fasten the delicate wires from the secondary coil into the web of the right foot. Arrange the inductorium for a tetanizing current and find a strength of stimulus which will cause reflex movements of the right leg.

Now determine the reflex time when 0.2 per cent sulphuric acid is applied to the left foot by Türk's method. Wash off the acid, wait a few minutes, and then repeat the observation, taking the average of the two.

Now close the key in the primary circuit of the tetanizing current, and just as the short-circuiting key of the secondary current is opened to stimulate the right foot, again immerse the left foot in the 0.2 per cent sulphuric acid as before, and determine the reflex time. Repeat this observation.

Observe that reflexes are inhibited, or the reflex time is prolonged, when stimuli reach the spinal centers simultaneously from different parts of the body, i. e., when nerve cells are occupied with one set of nerve impulses they are less sensitive to others. Record your results.

## TRACTS OF THE SPINAL CORD.

**219. Crossed Pyramidal Tract:** Study the section of a human spinal cord showing secondary degeneration of the crossed pyramidal tract, or according to the new nomenclature,



the fasciculus cerebrospinalis lateralis, at different levels of the cervical and thoracic cord. The specimens have been stained by a modification of Marchi's method.

**220. The Great Pyramidal Pathway in the Dog:** The brain and spinal cord of a dog were taken out two weeks after the removal of the Rolandic or motor area of the cerebral cortex; the tissues were fixed in Müller's fluid and stained by Marchi's method. The motor fibers, which have undergone secondary degeneration, are stained black by the osmic acid while the normal undegenerated fibers do not stain.

Sections have been made through the internal capsule, crus, pons, medulla and the cervical, thoracic and lumbar segments of the spinal cord. Study these sections carefully, and observe the position of the pyramidal fibers at each level; also the gradual diminution in the number of fibers as the crossed pyramidal tract extends down the cord.

Note especially the position of these fibers in the upper part of the medulla, the decussation of fibers at the level of the decussation of the pyramids, and the position of the crossed pyramidal tract at the lower level of the medulla.

**221. Secondary Degeneration from Hemisection of the Spinal Cord:** Lateral hemisection of the spinal cord of a cat has been made at the junction of the thoracic and lumbar segments. Sections have been made at different levels above and below the lesion and stained by Marchi's method.

Study these sections carefully. Observe the tracts which show ascending degeneration throughout the length of the cord, viz., (1) the fasciculus gracilis, formerly called the postero-median or column of Goll; (2) the fasciculus cerebellospinalis, or according to the old nomenclature, the direct cerebellar; and (3) the fasciculus anterolateralis superficialis, also known as the antero-lateral ascending or column of Gowers; also the tracts which only degenerate a short distance above the lesion, viz., the fasciculus cuneatus, formerly called the postero-external or column of Burdach, and the marginal zone of Lissauer.

Note the extensive degeneration in the anterior and lateral ground bundles immediately above and below the lesion.





Observe the descending degeneration in the fasciculus cerebrospinalis lateralis or crossed pyramidal tract below the lesion; also in the rubro-spinal tract.

**222. Secondary Degeneration After Section of the Posterior Column of the Spinal Cord:** The posterior columns of the spinal cord of a cat have been cut, in one case between the last thoracic and first lumbar segments, and in another animal, between the last lumbar and first sacred segments. The sections have been stained by Marchi's method.

Observe the degeneration in the fasciculus cuneatus or column of Burdach for a short distance above the lesion. The degeneration in the fasciculus gracilis or column of Goll extends up to the medulla, although the number of degenerated fibers is much less in the upper segments.

**223. Secondary Degeneration in the Spinal Cord From Section of the Posterior Nerve Roots:** The posterior nerve roots of the fifth and sixth lumbar nerves have been cut above the spinal ganglia on one side in a cat. The sections have been stained by Marchi's method.

Study these sections carefully. Observe degeneration of the fasciculus cuneatus or column of Burdach and the column of Lissauer just above the section. The fasciculus gracilis or column of Goll shows degeneration in some of its fibers on the same side up to the medulla, but the number steadily diminishes and is much less than that after section of the posterior columns of the cord.

## CEREBRUM, CEREBELLUM AND SEMICIRCULAR CANALS.

The sigmoid gyrus of the dog's brain is exposed by trephining. This corresponds to the Rolandic area of the human brain. It curves about the cruciate fissure which corresponds to the fissure of Rolando (sulcus centralis) in more highly developed brains.

Observe that stimulation of the sigmoid gyrus by a tetanizing current of suitable strength (i. e., the minimal current which will cause contractions) produces contractions of the muscles of the hind leg, fore leg, or neck, according to the part stimulated. The movements only occur on the opposite side of the body. They



are not co-ordinated and may consist of irregular, spasmodic contractions of groups of muscles. By using a very strong stimulus spasmodic contractions of the same side of the body may be produced by diffusion in the spinal cord.

After having localized the motor area by stimulation, this part of the cerebral cortex is removed by scooping out the cortical substance. The entire operation is done with surgical cleanliness and the wound is closed. As soon as the animal recovers from the anesthetic, note the temporary paralysis of the parts of the body whose cortical centers have been destroyed.

Observe the animal for the next few days and note that the paralysis rapidly disappears. The movements of locomotion are soon regained and the "deficiency phenomena" are only seen in some unusual movements of the body. The animal is able to walk and run without any permanent paralysis. Such remarkable recovery only occurs in the lower animals, where the activities of the spinal cord depend upon the flow of the afferent impulses from the periphery more than the impulses from the cerebral cortex.

**224. The Effects of Removing the Cerebral Lobes in the Frog:** The cerebral lobes in several frogs have been removed on the previous day. The optic tracts, optic lobes, medulla and spinal cord are intact.

Observe (1) that the posture is the same as that seen in a normal frog and quite different from that of the "spinal" or reflex frog; (2) the croaking reflex of the male frogs is present; (3) the "balancing" reflex from gradually changing the base of support is preserved; (4) all the movements of the body are co-ordinated and performed as perfectly as in a normal frog so that no paralysis can be detected; (5) the frog avoids opaque objects in hopping; (6) not only is the frog able to maintain body equilibrium, but it swims perfectly when placed in water; (7) the frog does not move spontaneously, but only in response to some form of stimulus.

**225. The Effect of Removing the Cerebral Lobes in a Pigeon:** The cerebrum of a pigeon has been removed two hours before the demonstration. Observe that (1) the appear-



ance is different from that of a normal bird; (2) the animal balances perfectly on a perch and regains its balance when disturbed; (3) the animal walks and flies when compelled to do so, showing that it can maintain body-equilibrium and perform the most complex co-ordinate movements of which it is capable; (4) there is no spontaneity of movement and the animal remains quiet when undisturbed.

**325. The Effect of Injuring the Cerebellum in a Pigeon:** The animal has had its cerebellum destroyed two hours before the demonstration. Observe that the dullness and inactivity which result from removal of the cerebrum are absent in this pigeon. The pigeon can perform complex movements and does so spontaneously, but co-ordination is disturbed and at times the animal has difficulty in maintaining body equilibrium. At times forced movements may occur, i. e., activities which seem to be beyond the control of the will.

**227. The Effects of Destroying the Semicircular Canals in Frogs:** These are destroyed by introducing a pithing-pin into the temporal bone. Note that the frog can not maintain the normal posture, but turns the head towards the injured side. Observe that these frogs can not hop or swim as normal animals do, but perform circular or spiral movements. Co-ordination of the movements of the body is completely lost.

**228. The Effects of Destroying the Semicircular Canals in Pigeons:** (1) Observe the forced movements and lack of co-ordination in a pigeon which has had all the semicircular canals destroyed on one side.

(2) Describe the movements in a pigeon whose external (horizontal) semicircular canals have been destroyed on both sides.

(3) Describe the movements and the way a pigeon falls after destruction of the superior or anterior vertical semicircular canal on one side and the posterior vertical canal of the opposite side.

(4) Describe the effect of destroying the posterior vertical semicircular canals of both sides.

192





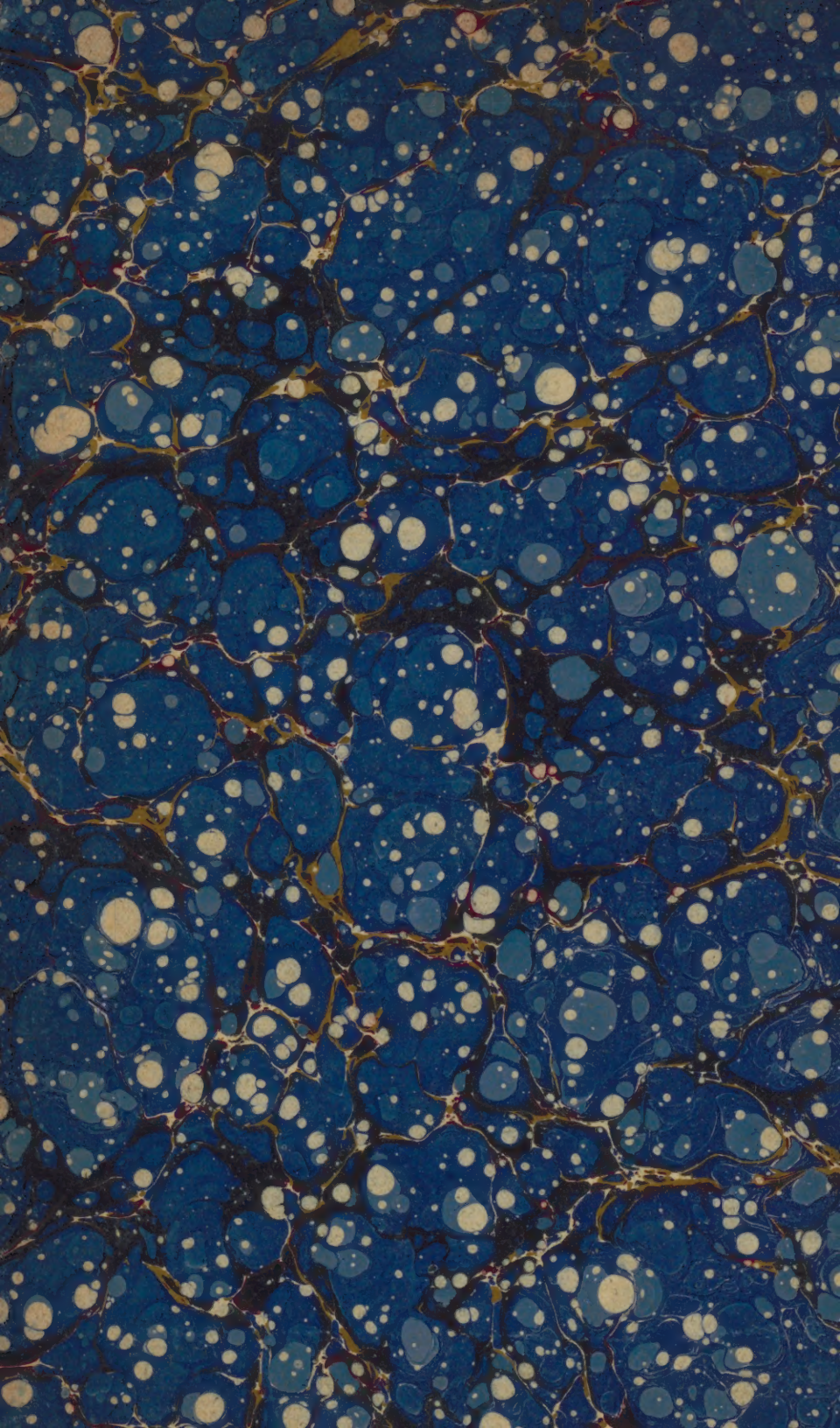




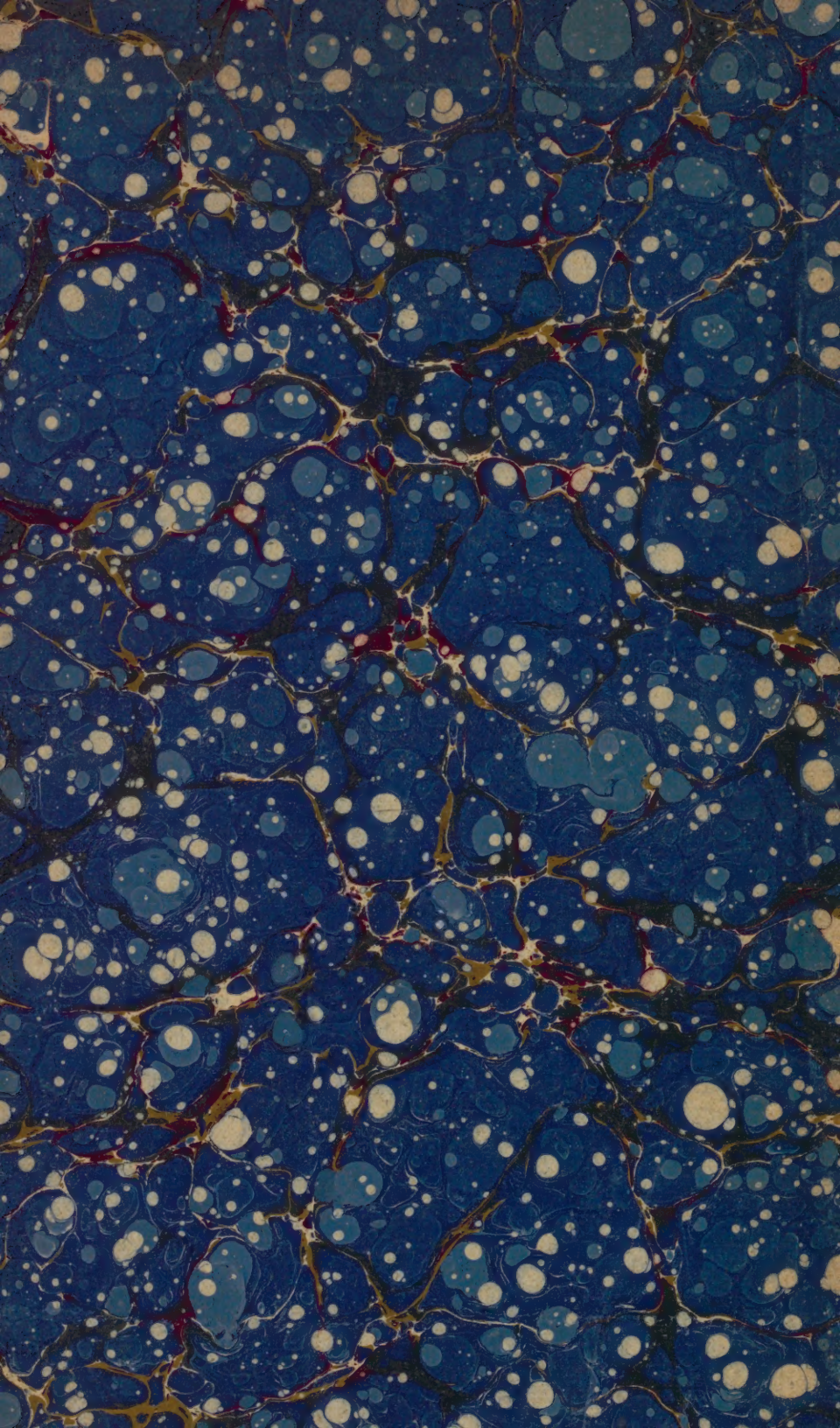














QT 25 C325L 1916

06920380R



NLM 05047656 3

NATIONAL LIBRARY OF MEDICINE